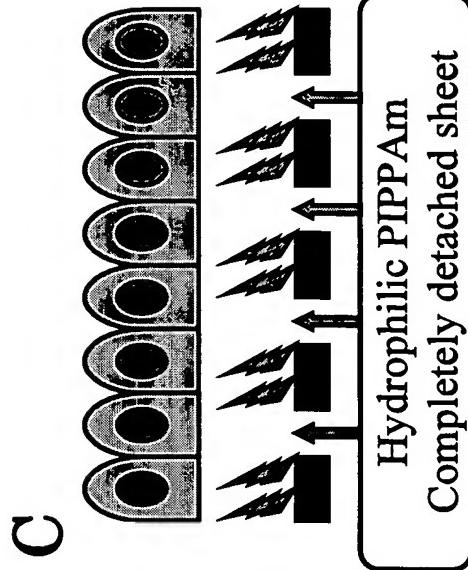
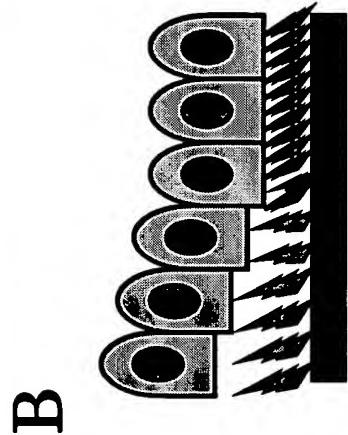
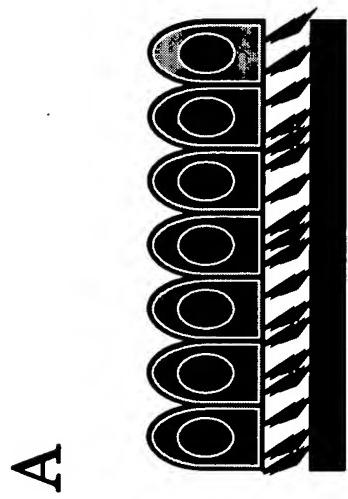
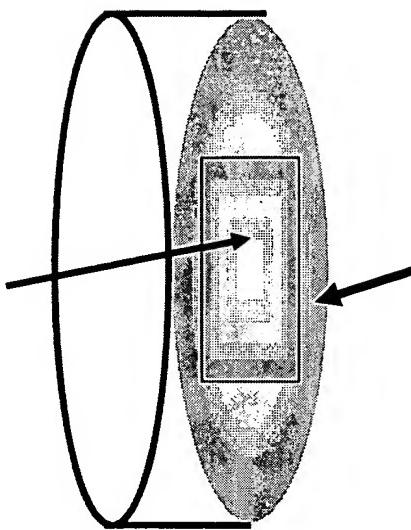


FIG. 1A

Cell Sheet Constructs



Temperature responsive polymer
Poly (N-isopropylacrylamide) (PIPAAm)



Exemplary Dimensions:

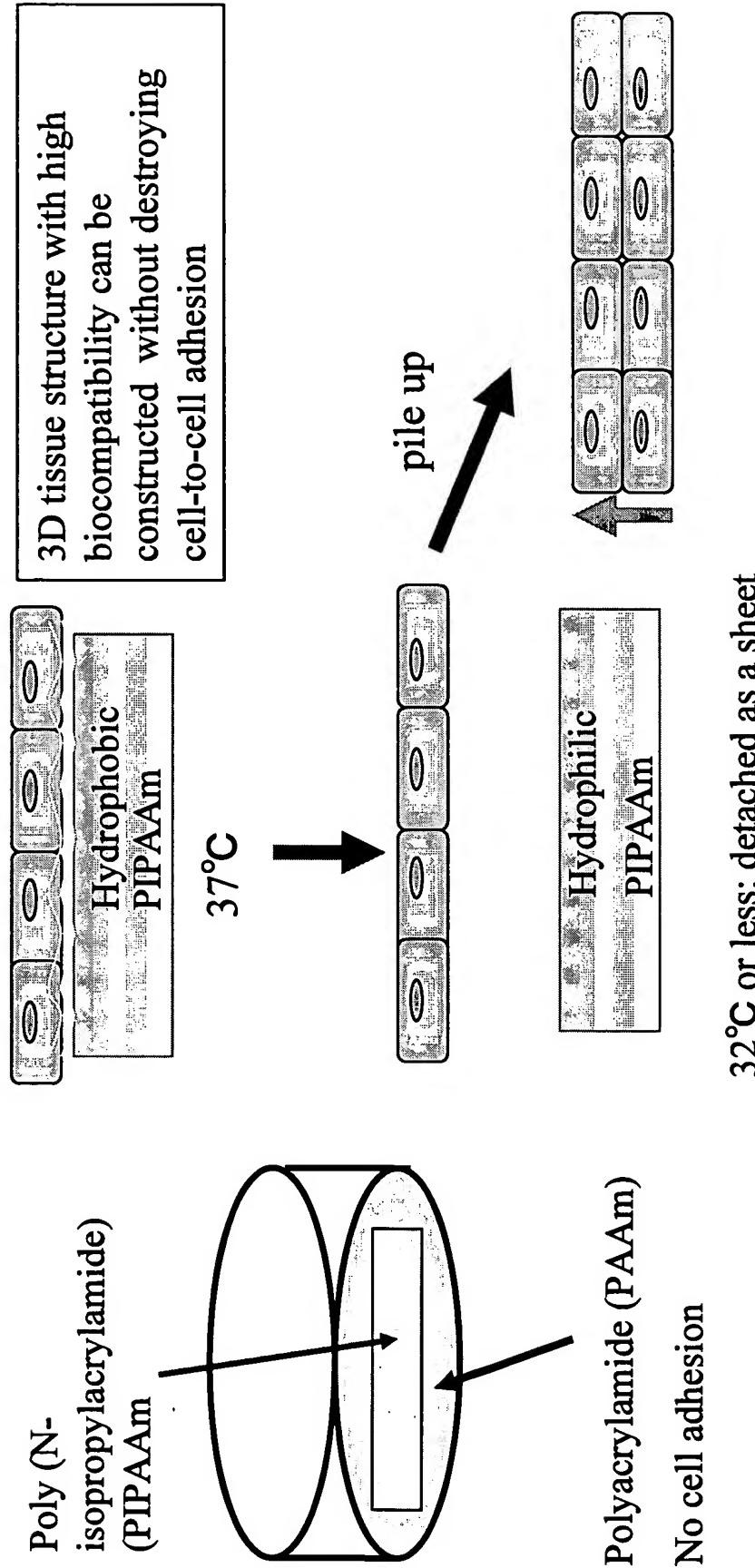
$1.11 \pm 0.05\text{cm}^2$ in area
 $50.2 \pm 6.0\mu\text{m}$ thick

BEST AVAILABLE COPY

Poly (acrylamide) (PAAm)

FIG. 1B

Temperature responsive culture dish



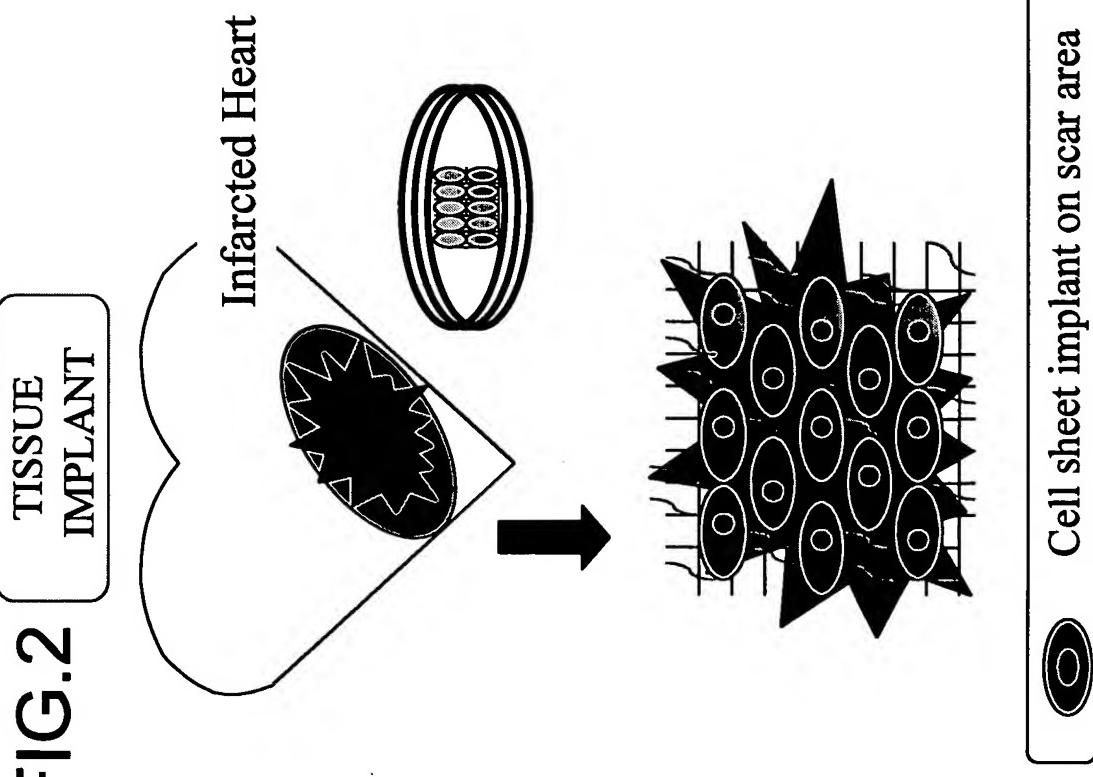
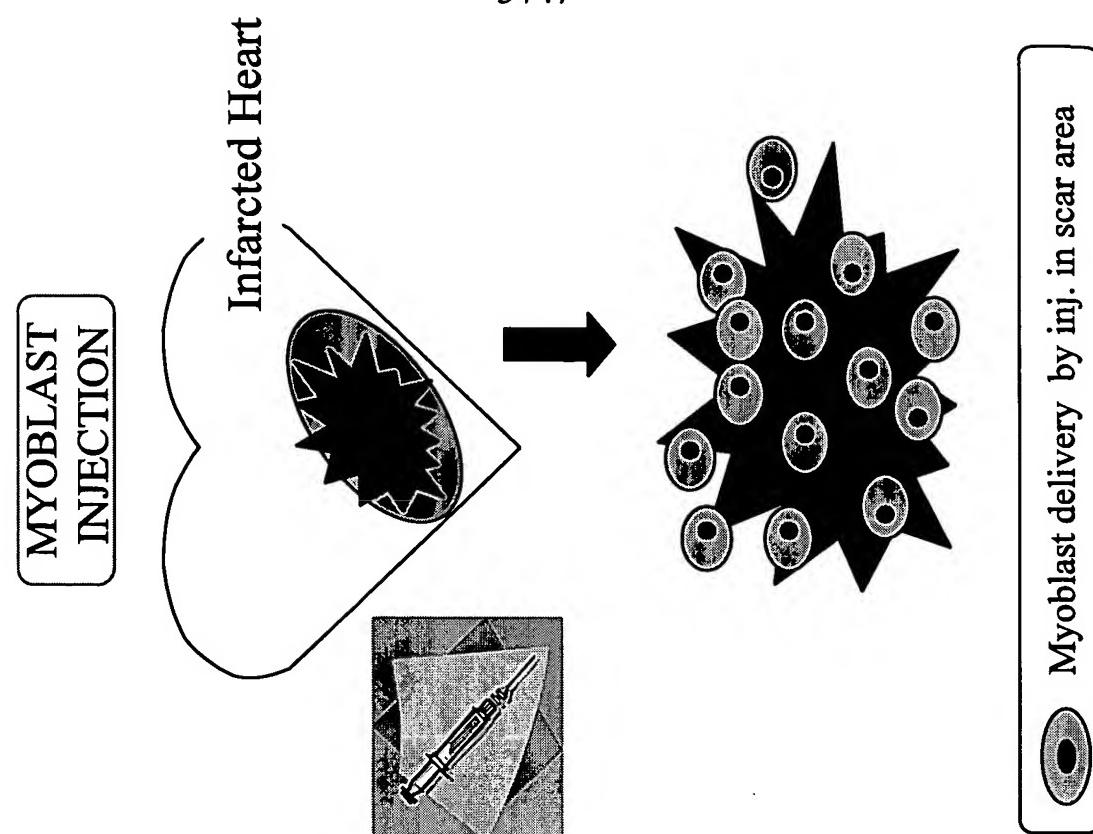


FIG.2

FIG.3 Experimental Protocol

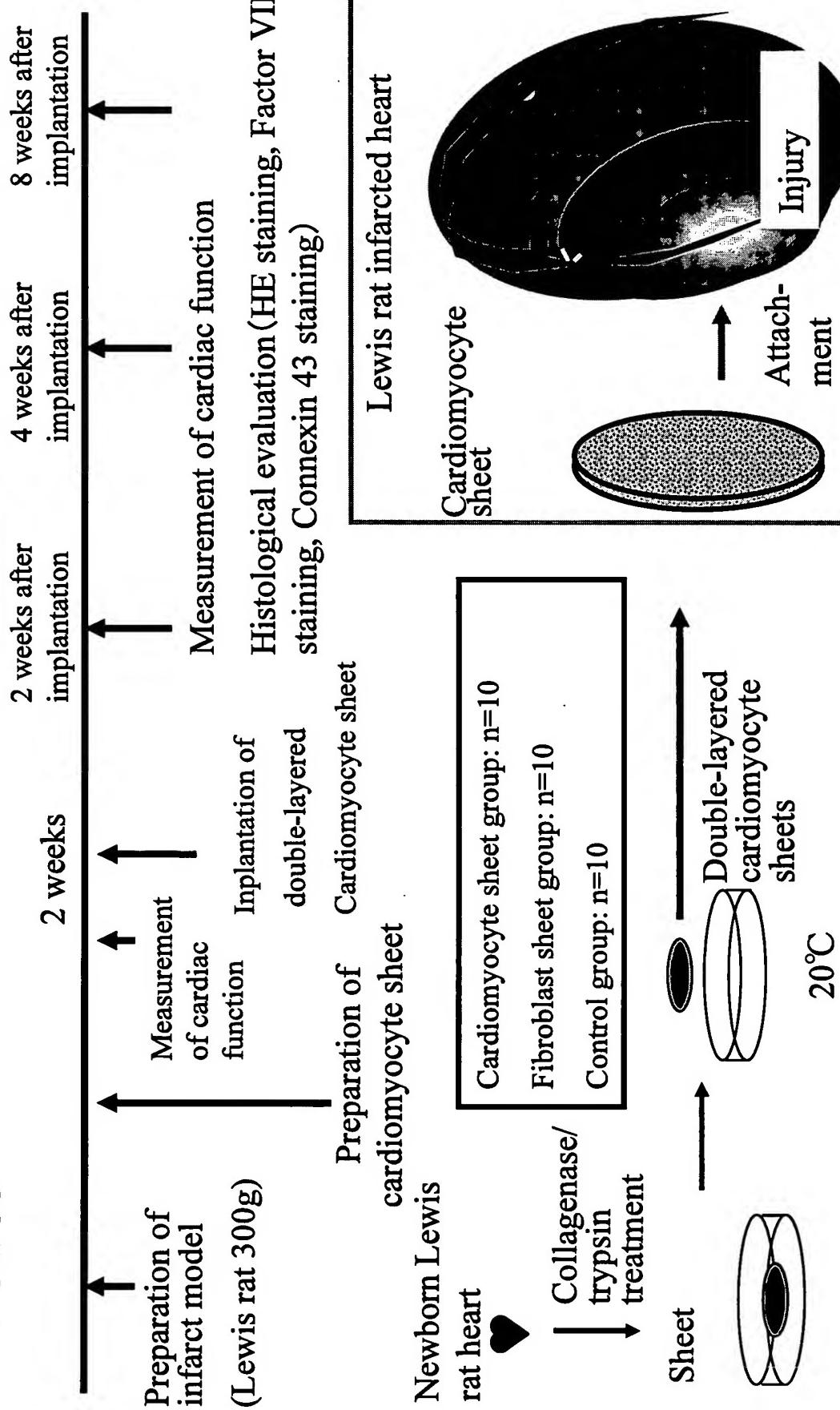


FIG.4 Regenerative therapy for cardiac muscle by cell transplantation

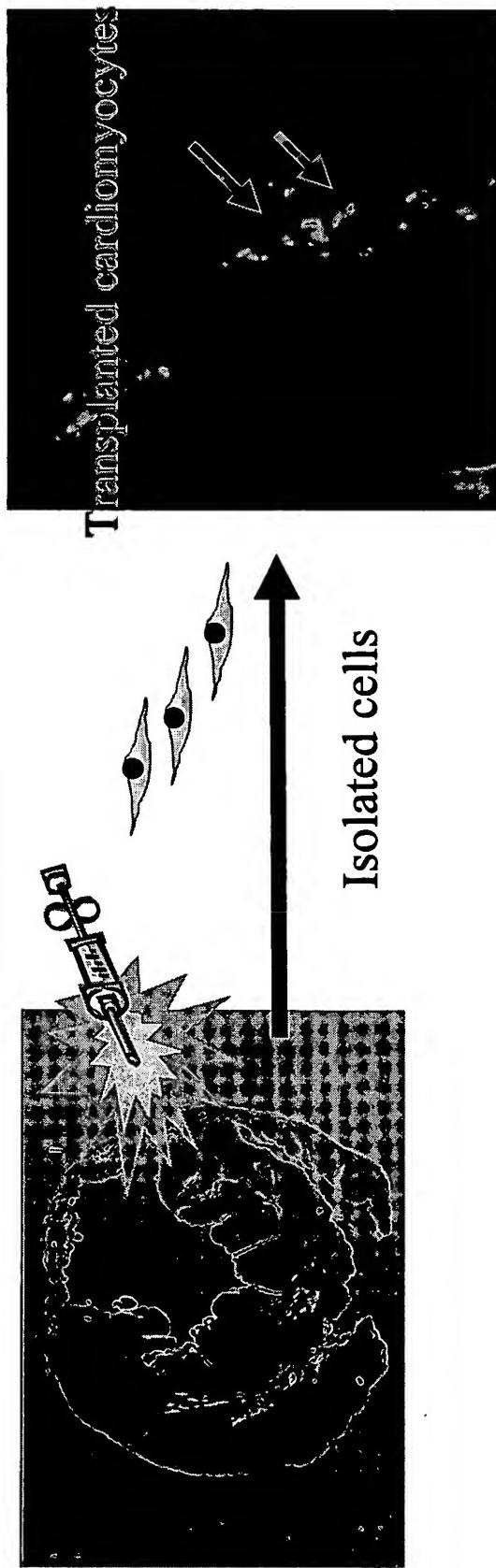


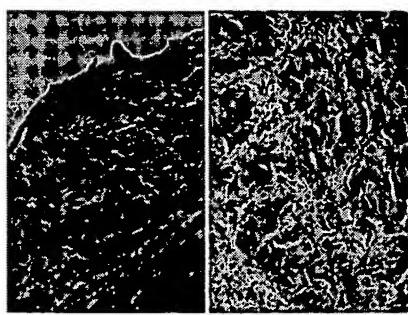
FIG.5 Problems with tissue transplantation

Cardiac muscle graft with scaffold

Alignment and cell-to-cell adhesion of transplanted cells within scaffold

Changes in scaffold in organism: elicitation of inflammation

Acceptance of scaffold by recipient's heart

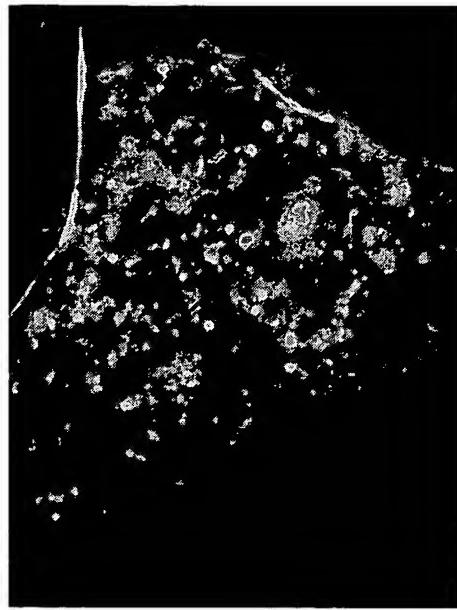


Development of high biocompatible cardiac muscle graft without scaffold

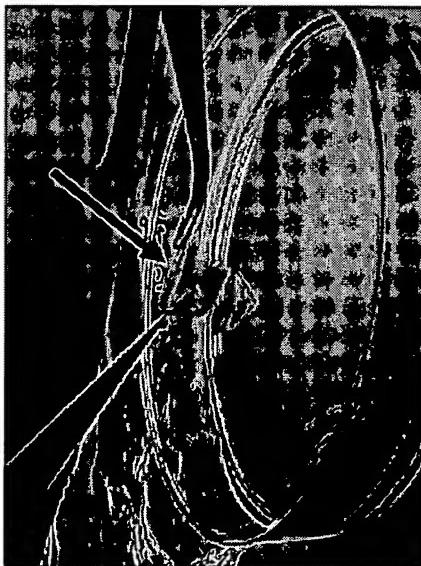
FIG.6 Implantation of cardiomyocyte sheet into infarcted heart



Implantation into rat infarct model



In vivo
Implantation of GFP rat newborn cardiomyocyte sheet



Cardiomyocyte sheet

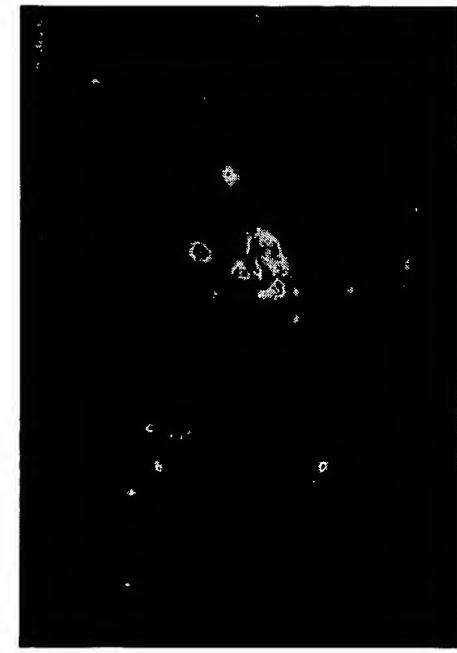
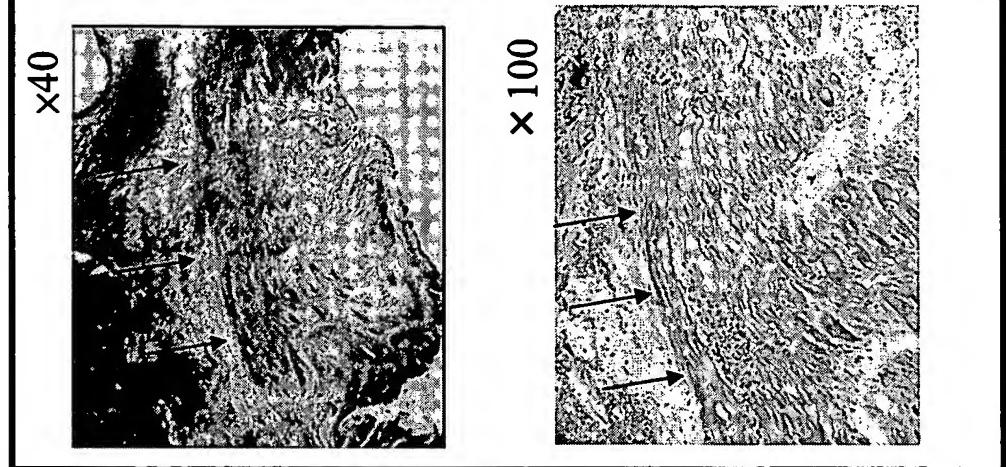
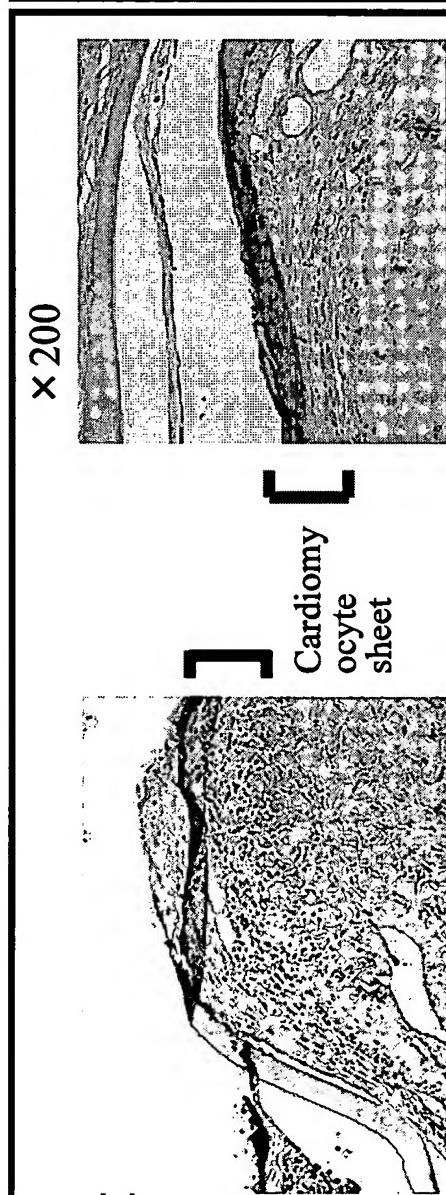
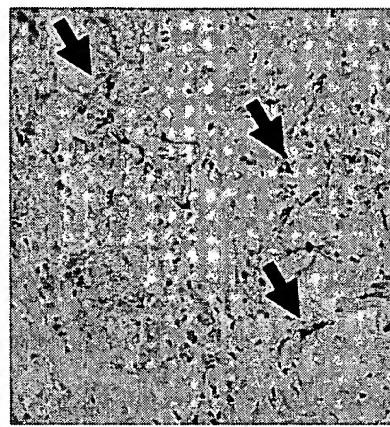


FIG.7

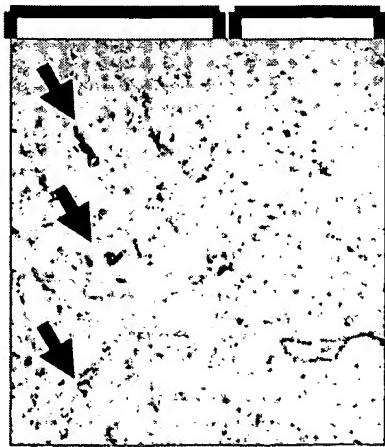
Tissue
2 weeks after implantation



HE staining

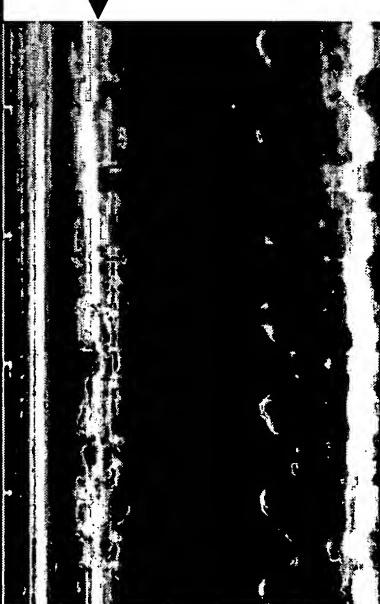
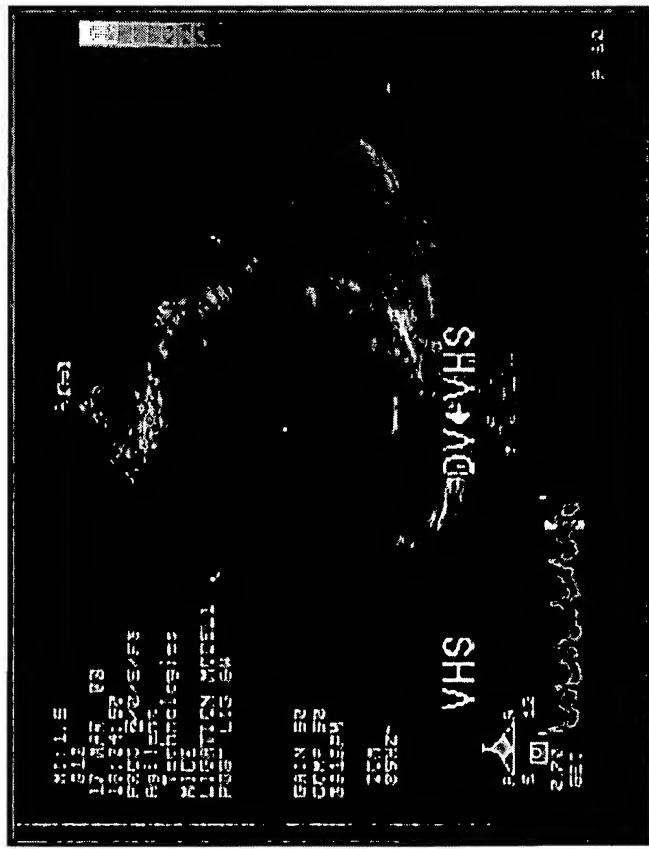


Factor VIII staining



Evaluation of Cardiac function - 1

Control



Implantation of prosthetic tissue

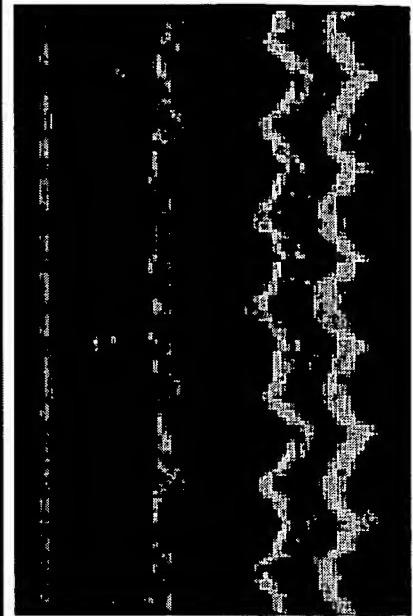
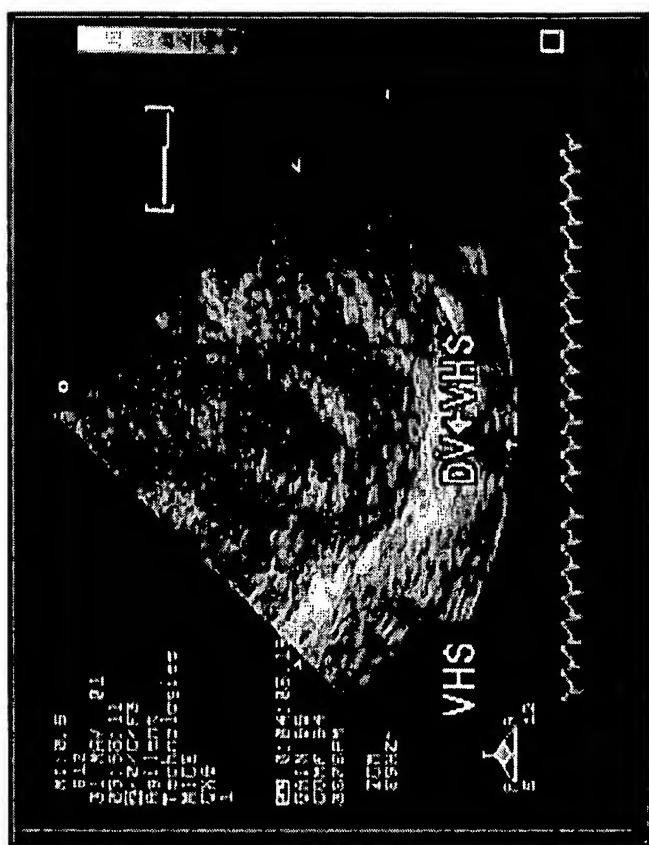
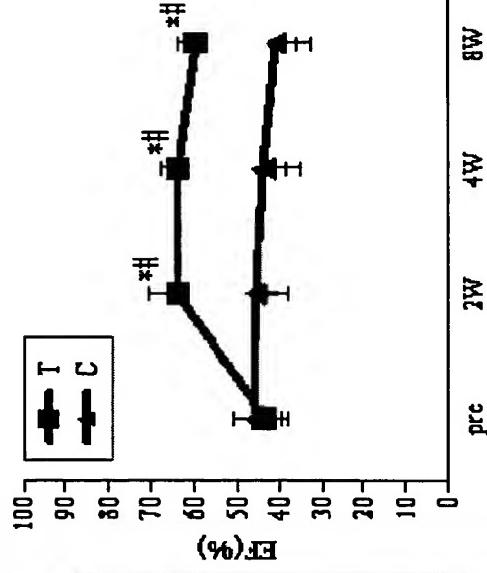


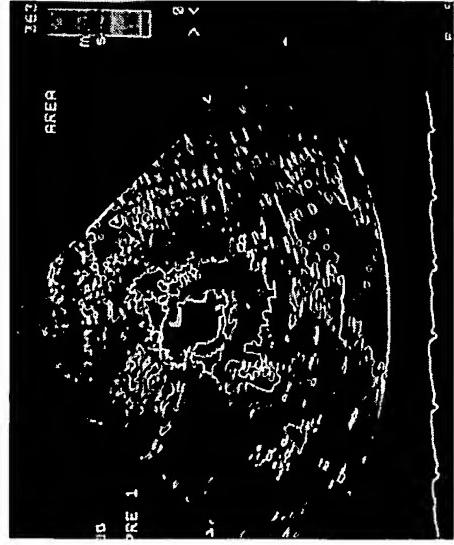
FIG.9

Evaluation of cardiac function - 2

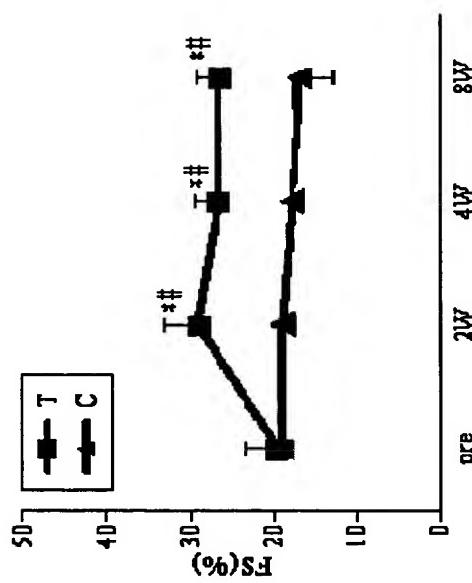
Ejection Fraction



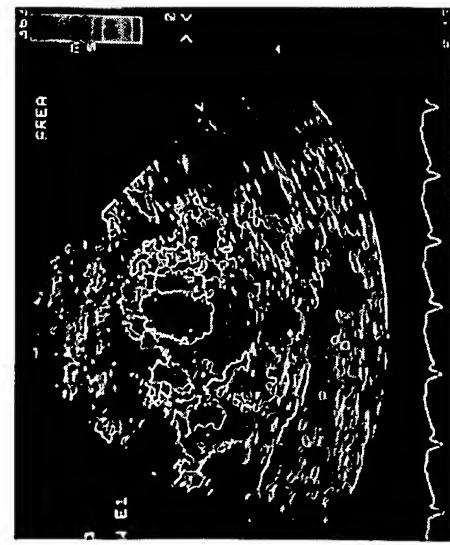
Base line



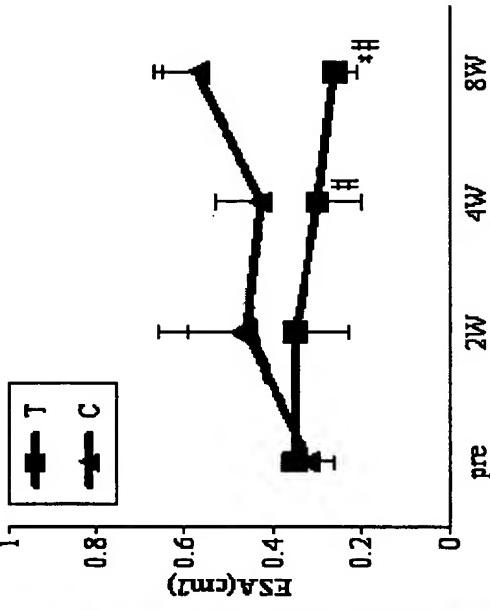
Fractional Shortening



Implanted cardiomyocyte sheet



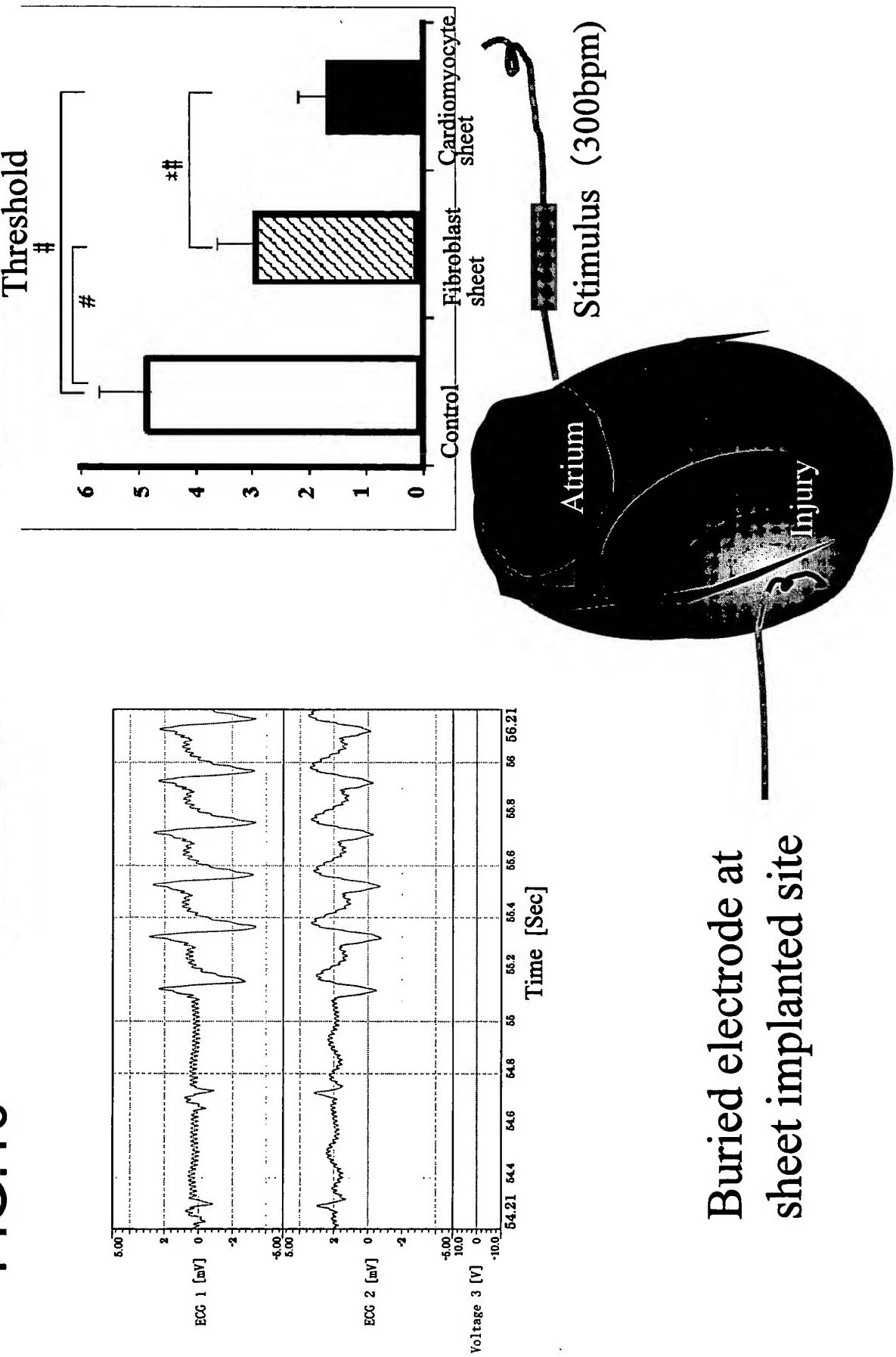
End-systolic area



*: p<0.05 to control

FIG. 10

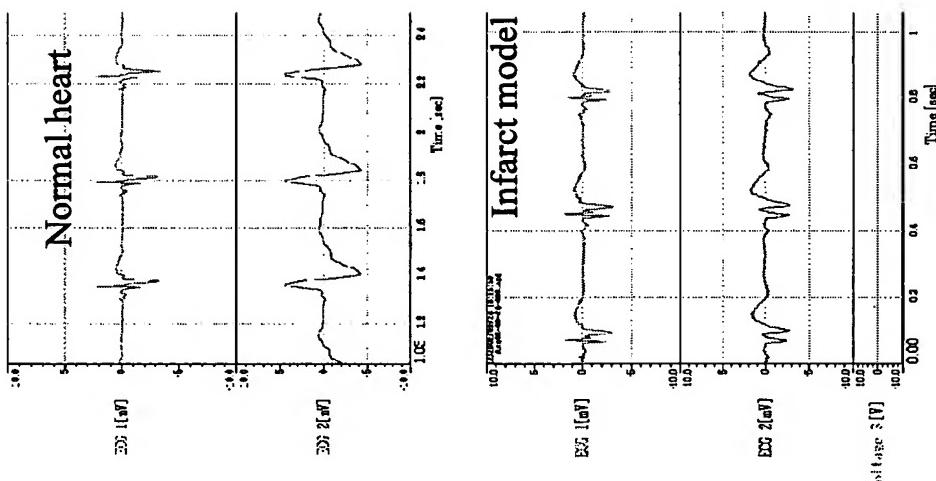
Electrophysiological Evaluation



Buried electrode at
sheet implanted site

Electrophysiological Evaluation

FIG. 11

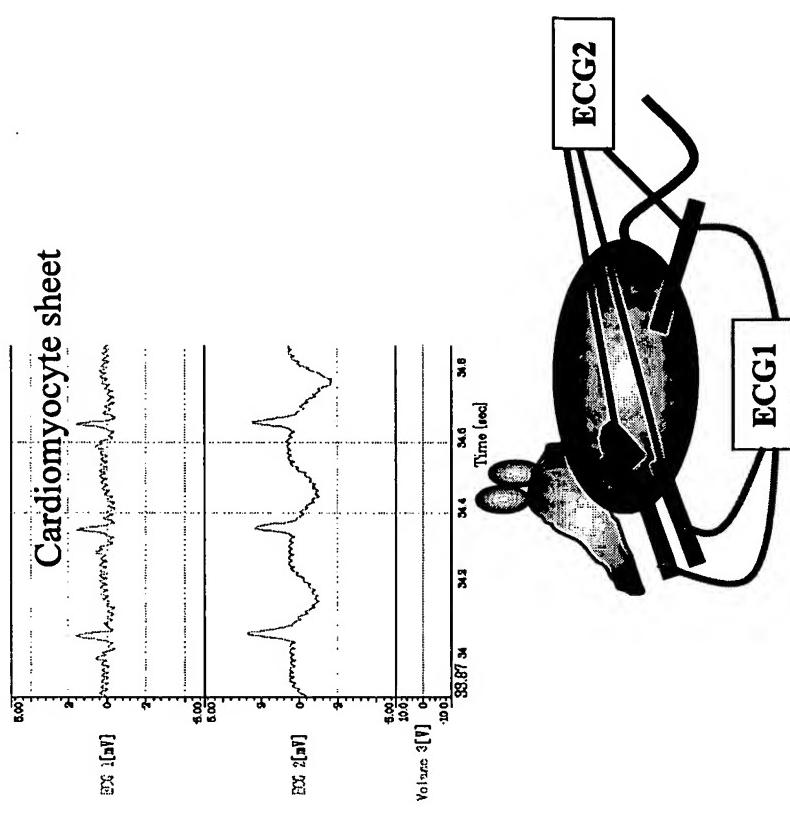


ECG 1:ECG (Surface)

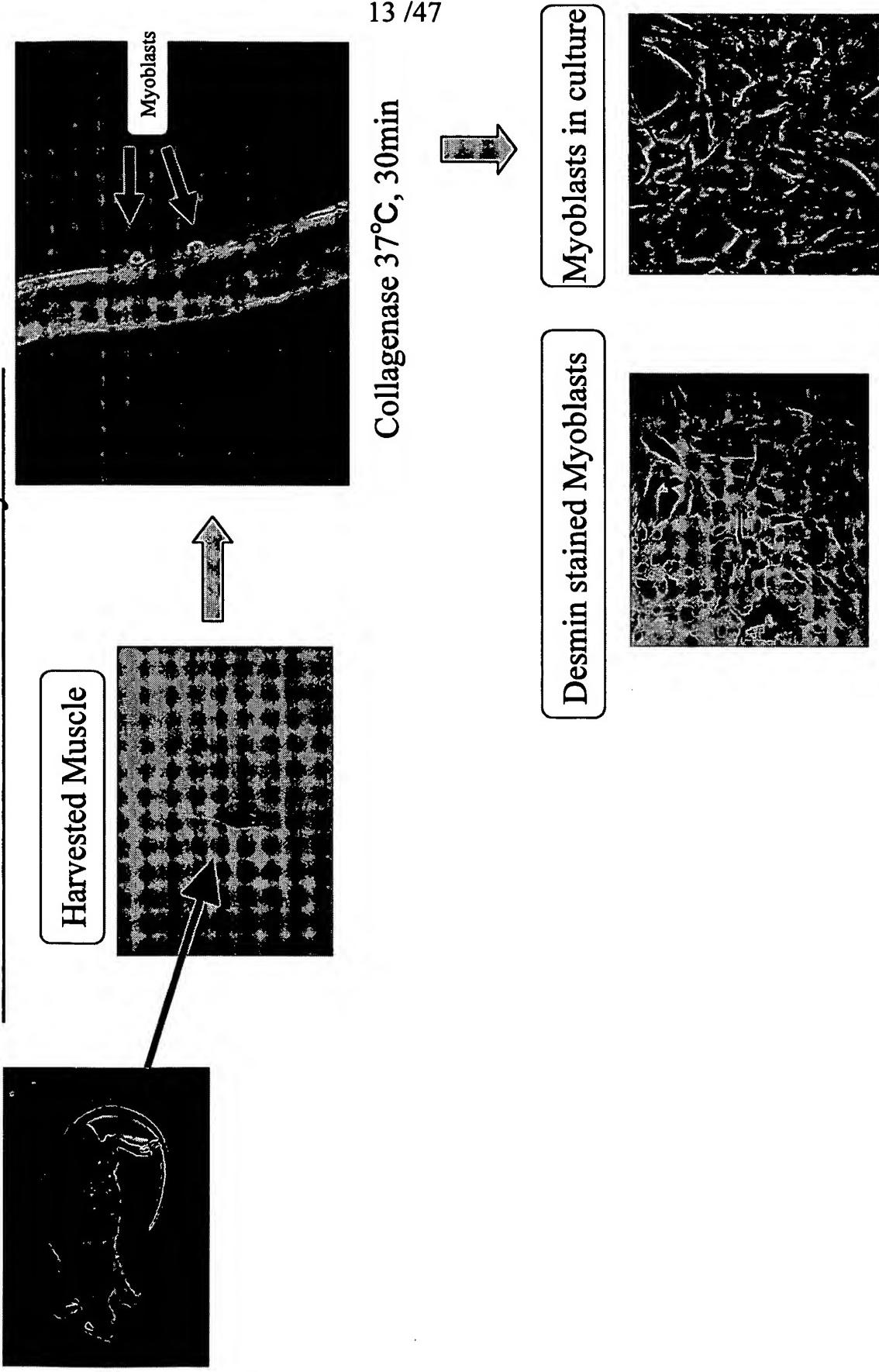
ECG 2: Normal heart (anterior wall)

Ligation model (injured)

**Prosthetic tissue implanted
(prosthetic tissue injured)**



Isolation and culture of myoblast



Methods: Myoblast Sheet Construction

FIG. 13

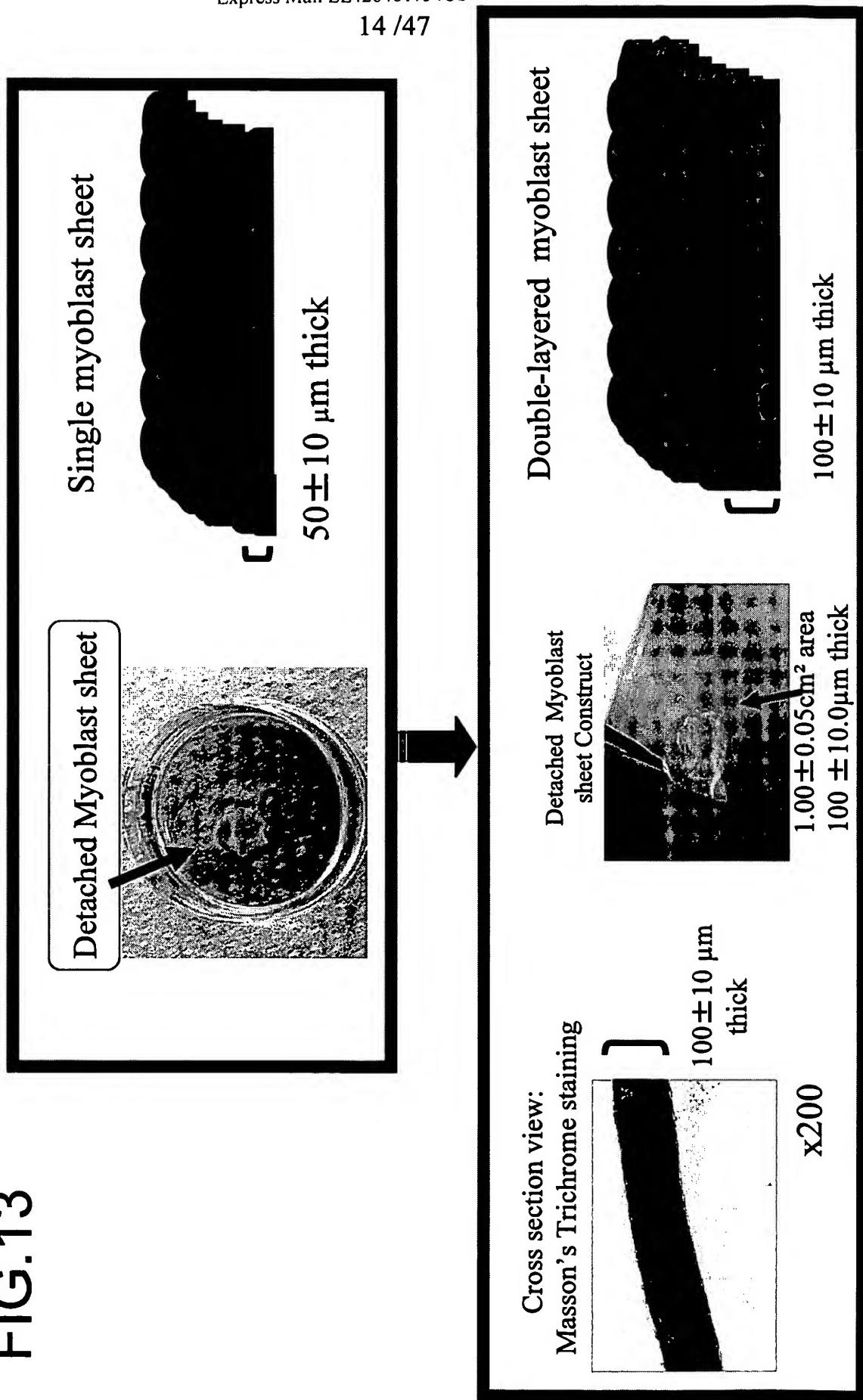


FIG. 14

Experimental Protocol

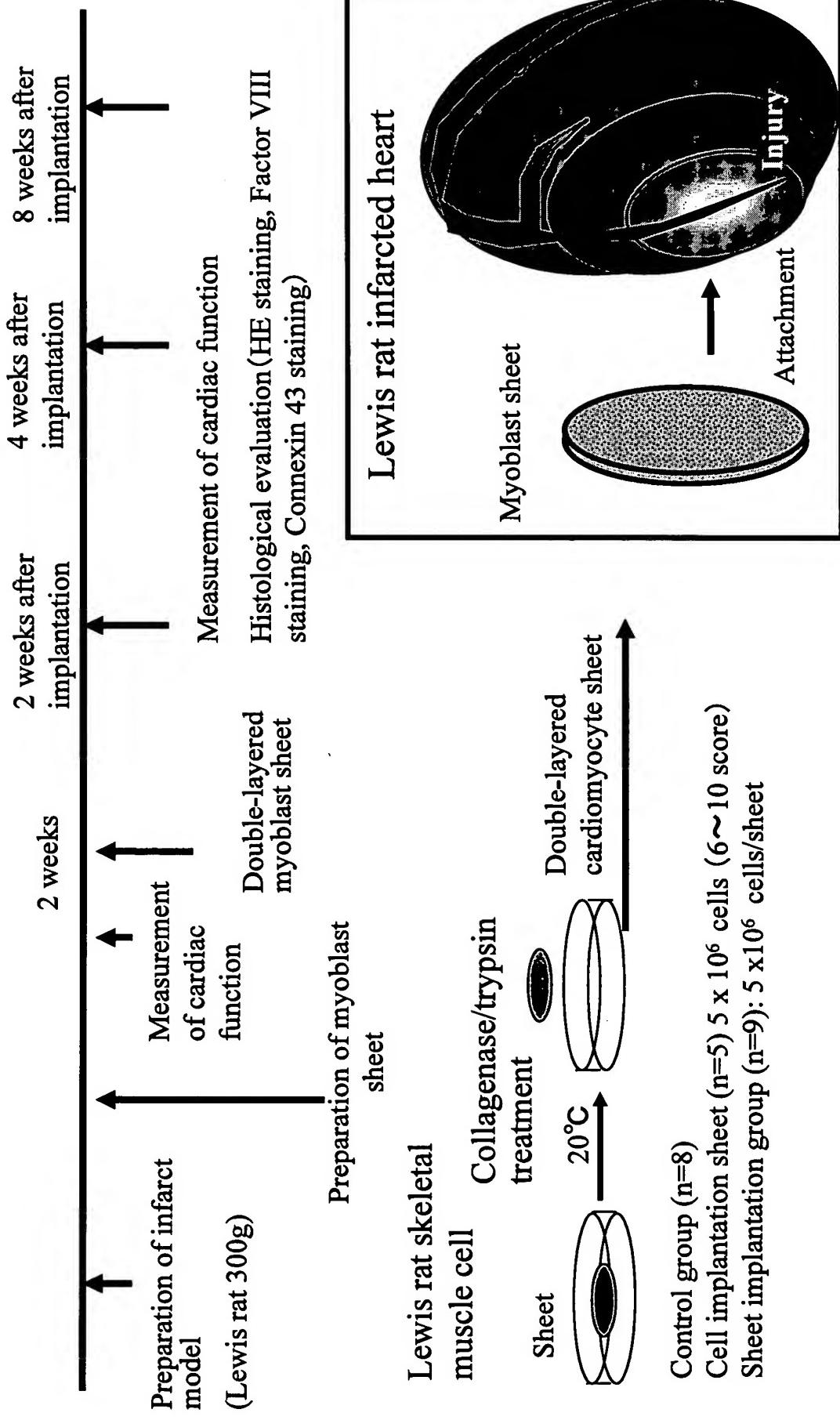
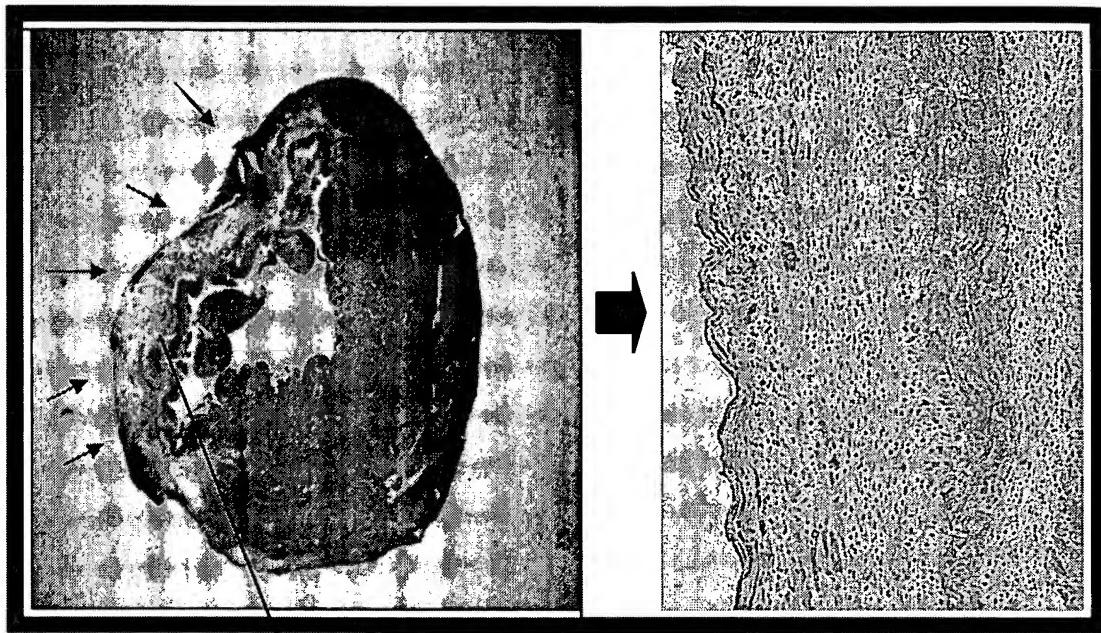


FIG. 15
Myoblast sheet: 4W post implantation



x10

x200

HE staining

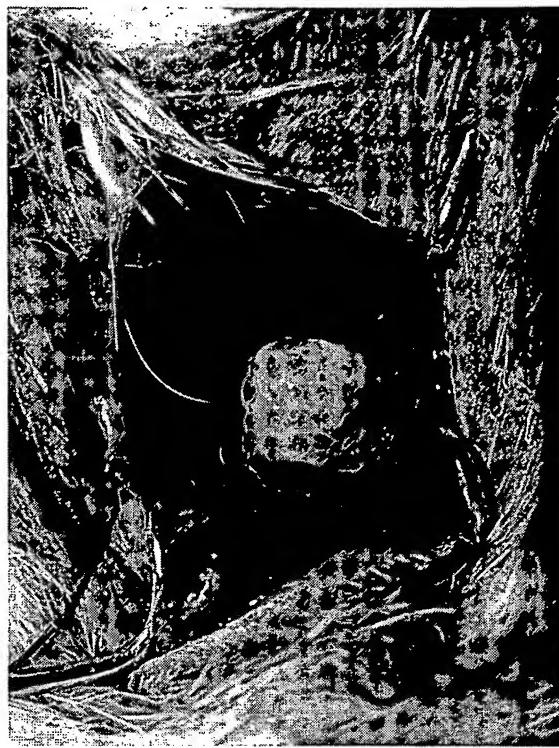


Implanted myoblasts

x1000

FIG. 16 Myoblast sheet Implantation procedure

After myoblast sheet implantation



Lewis rat ligation model

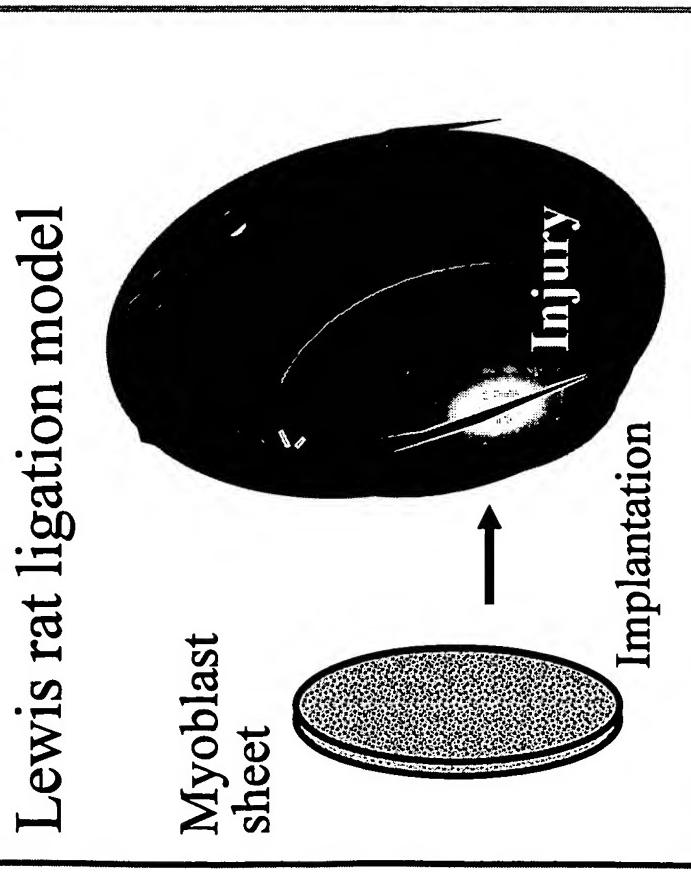


FIG.17 HistologyMasson's Trichrome Staining

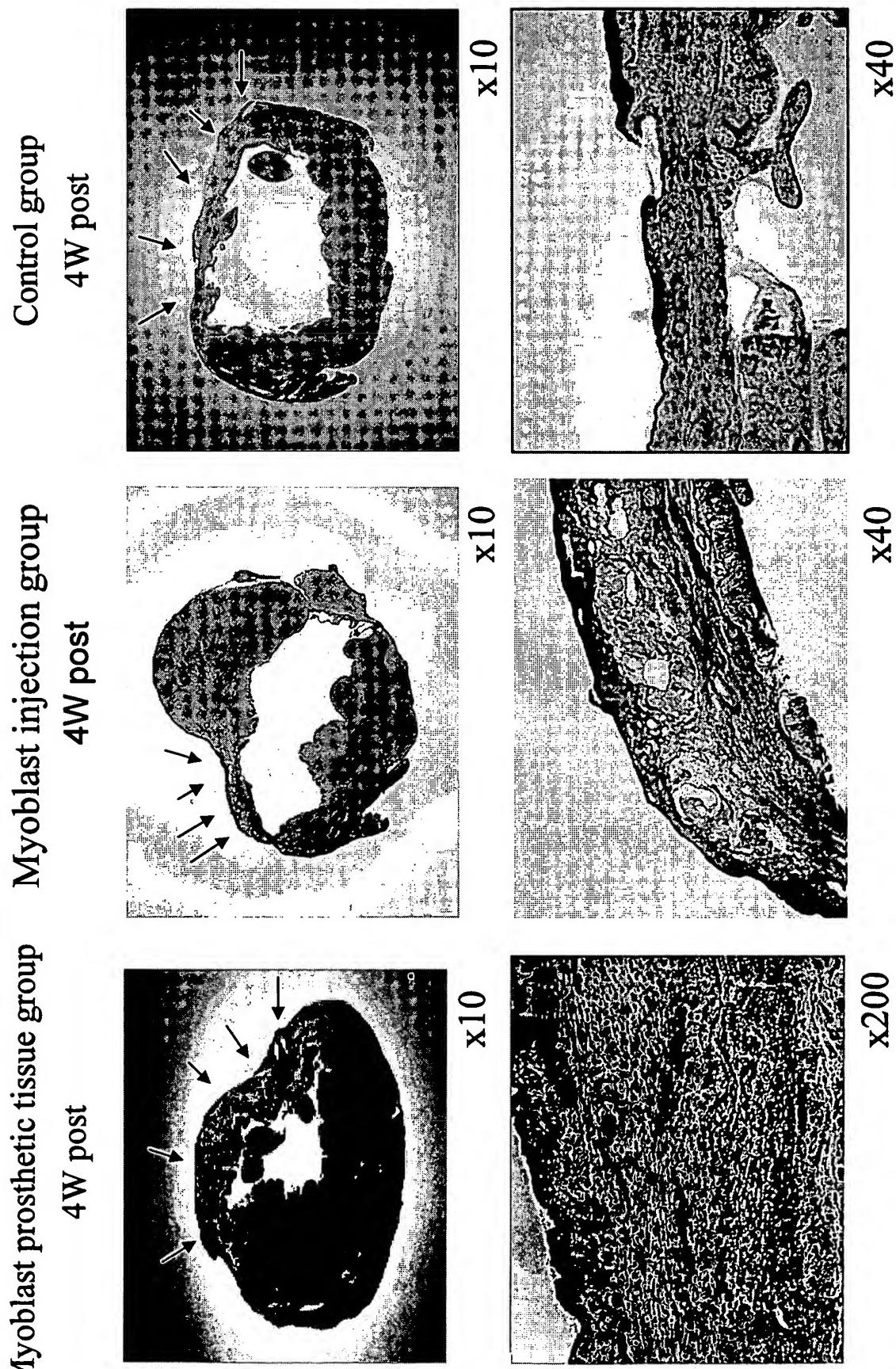
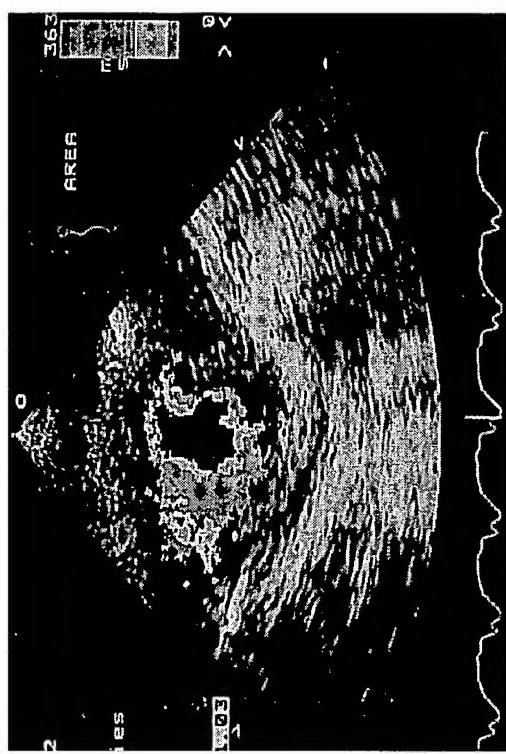
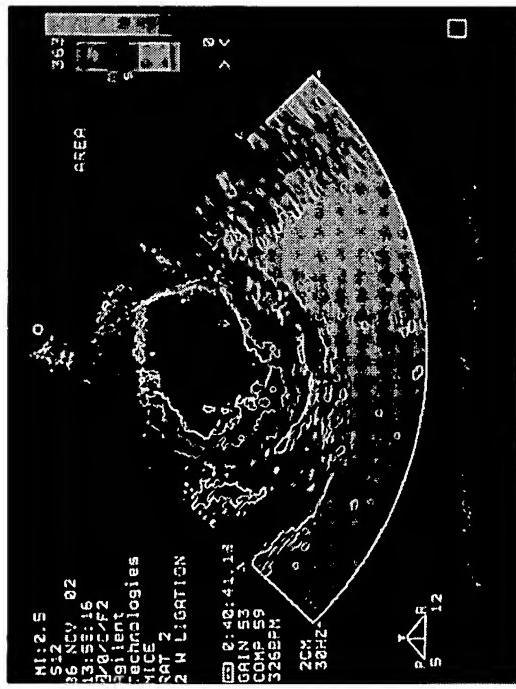


FIG. 18

CKA



M-mode analysis

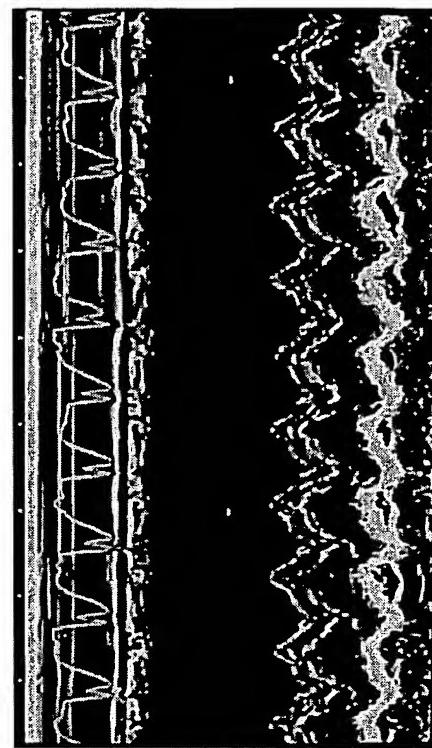
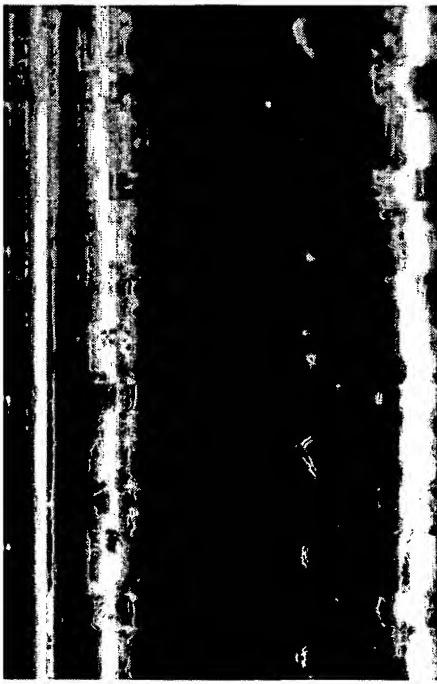
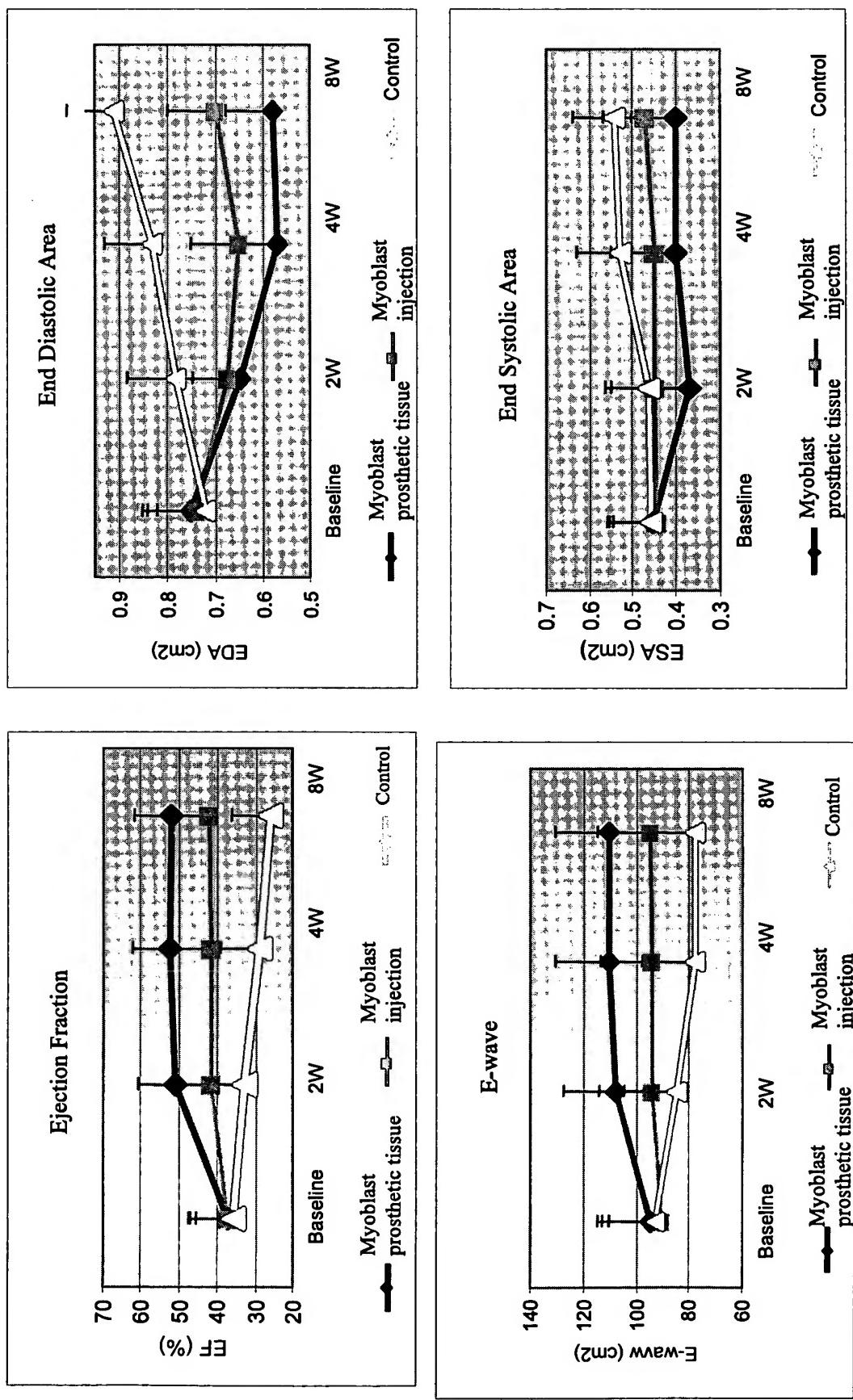


FIG.19



#P< 0.05 for control; *P< 0.05 to for injection needle group

FIG.20 Anterior Wall Thickness Comparison
Myoblast injection



x40

Control



x40

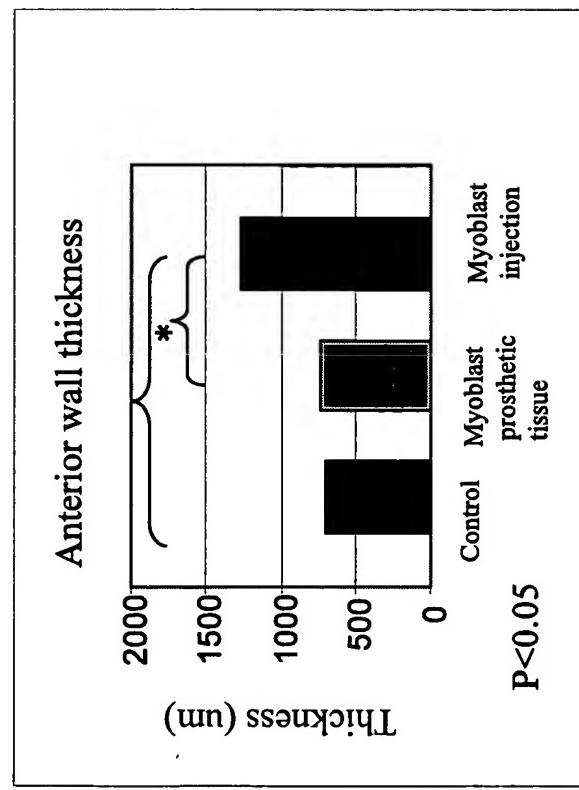
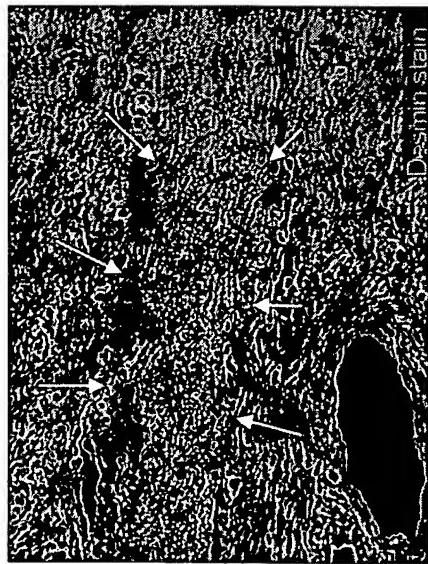


FIG.21 Myoblast sheet:
Desmin Staining



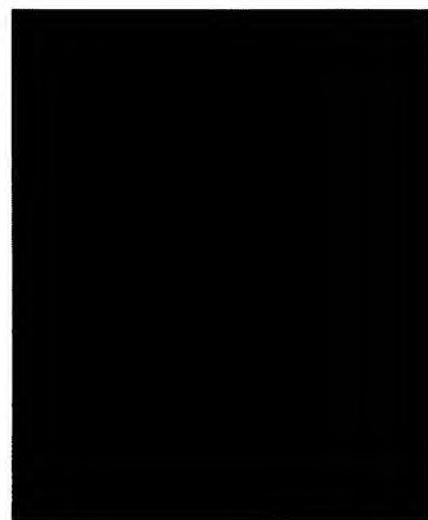
x100

Control



x40

Myoblast prosthetic
tissue group (GFP)



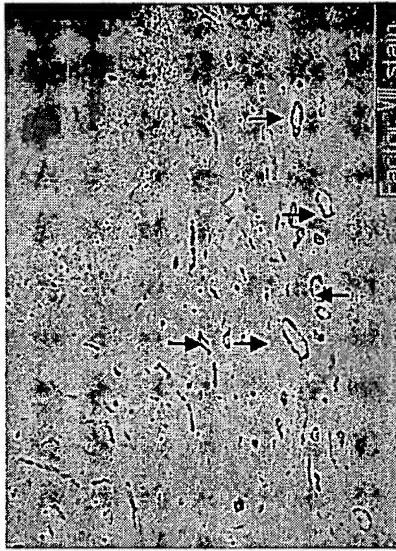
x100

Factor VIII staining



x40

Myoblast prosthetic tissue



22 /47
x100

Myoblast prosthetic tissue

x40

FIG.22A

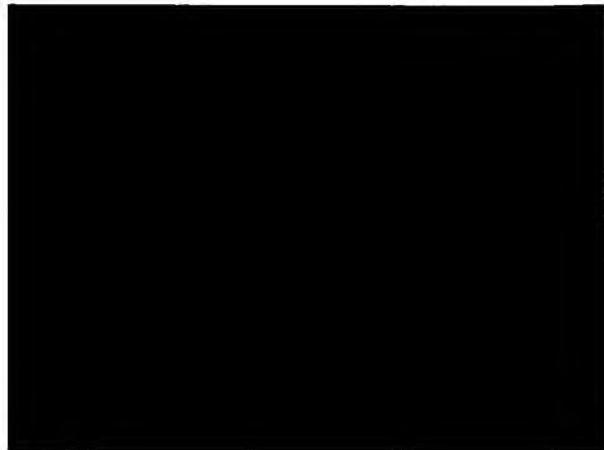


FIG.22B

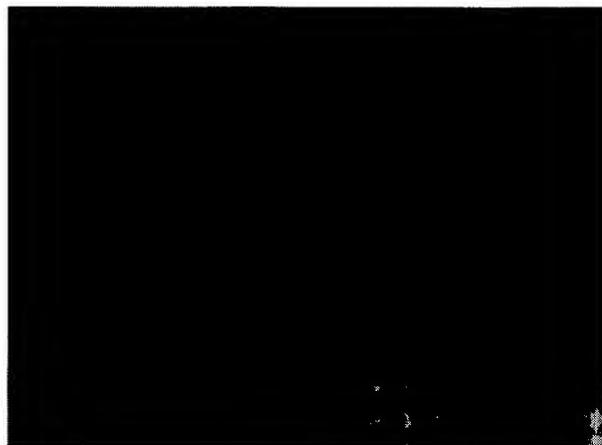


FIG.22C

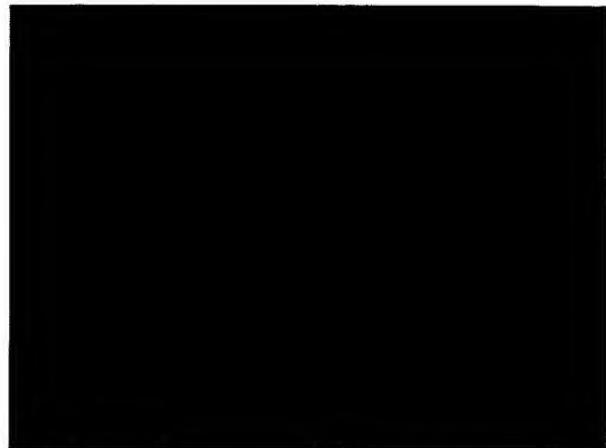


FIG.22D

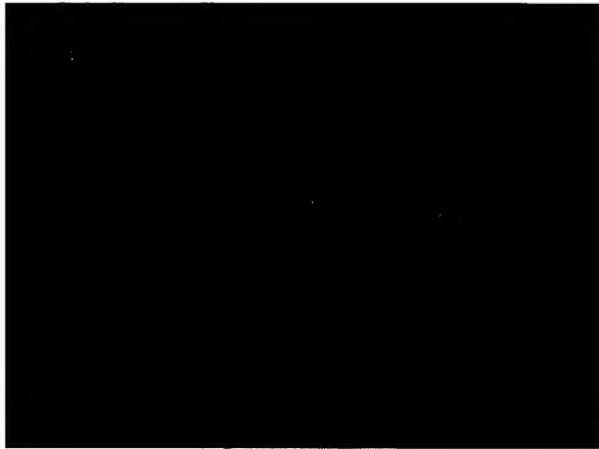


FIG.22E

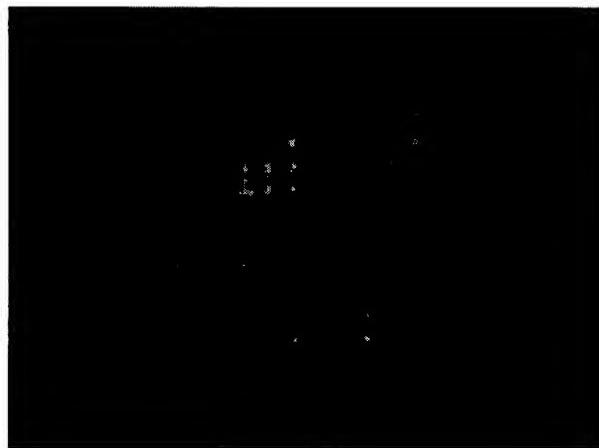


FIG.22F

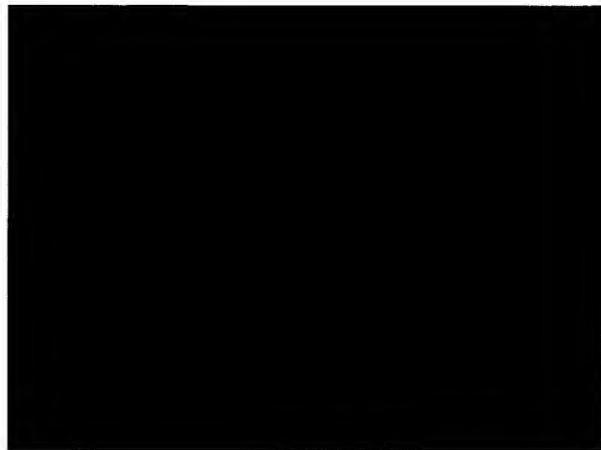


FIG.23A

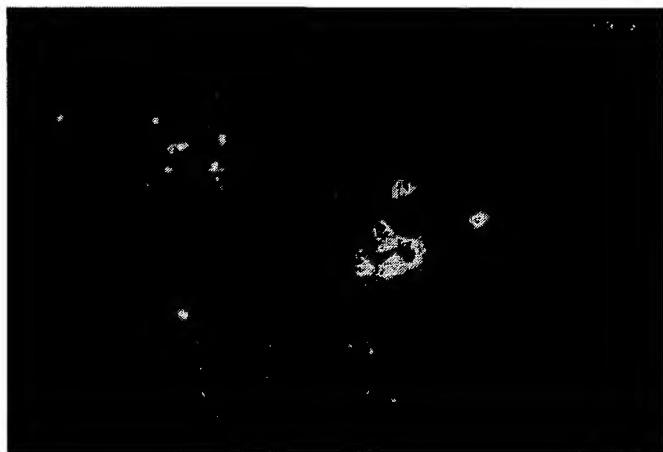


FIG.23B

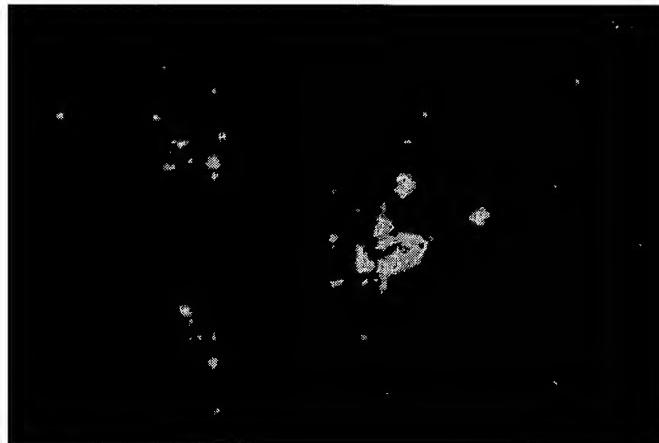


FIG.23C



FIG.24A

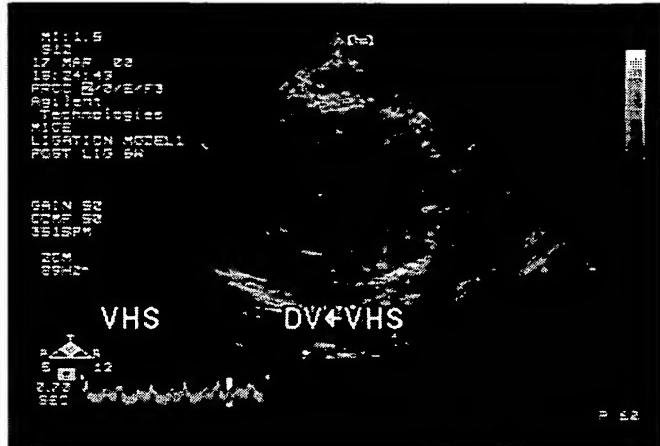


FIG.24B

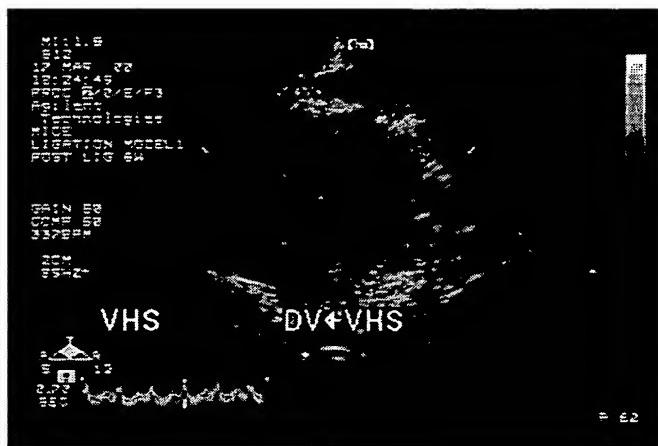


FIG.24C

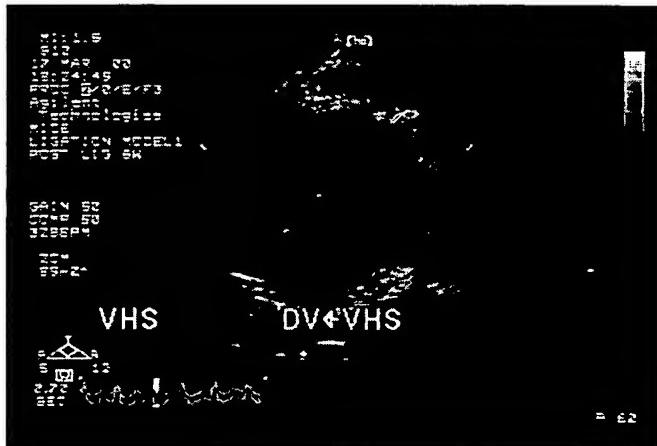


FIG.25A

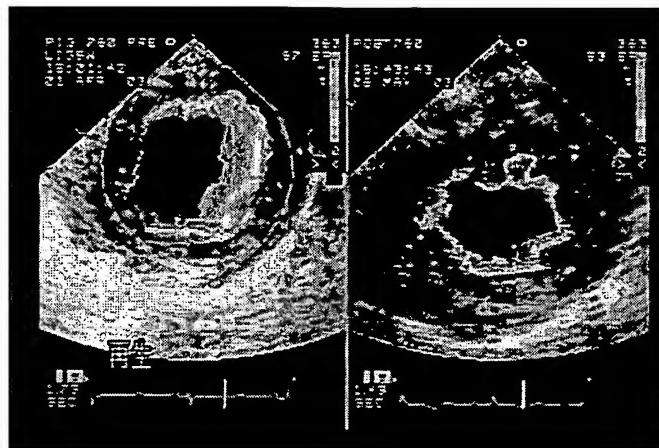


FIG.25B

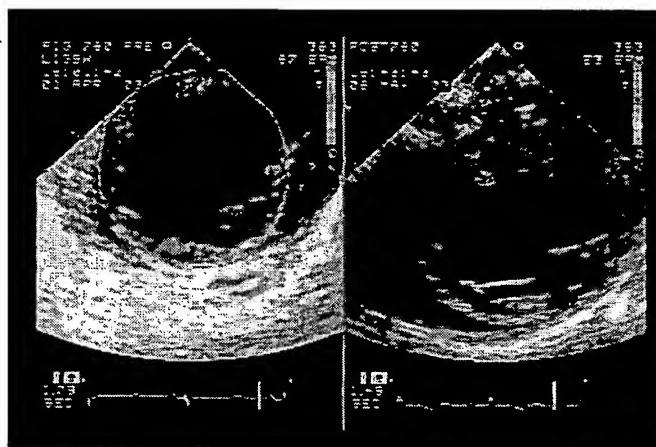


FIG.25C

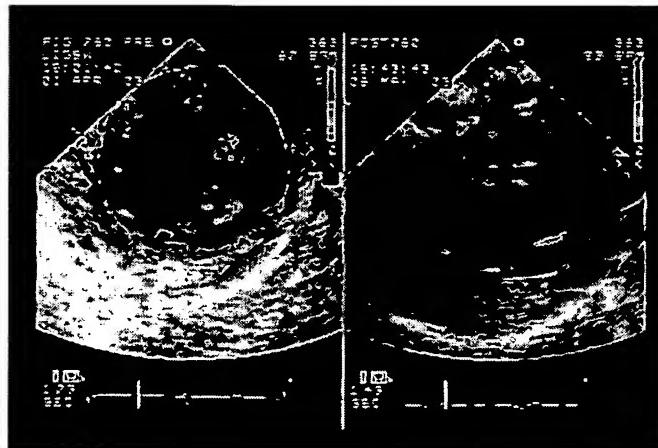


FIG.26A

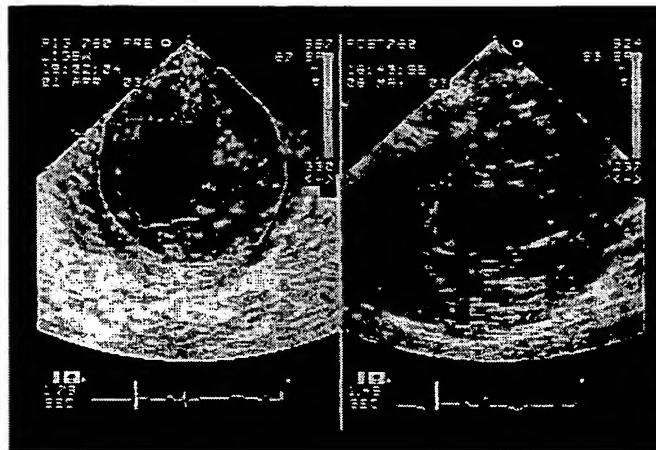


FIG.26B

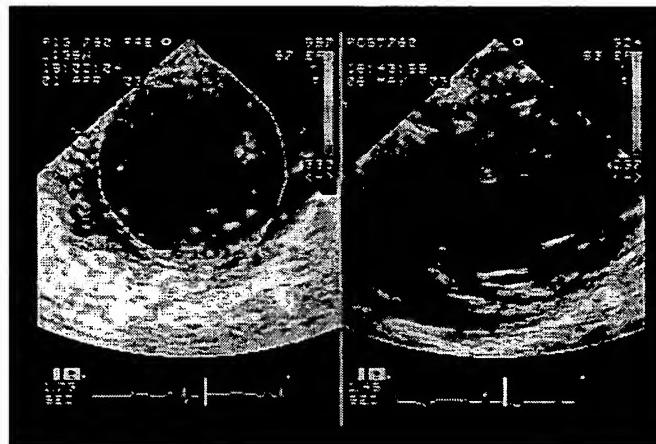


FIG.26C

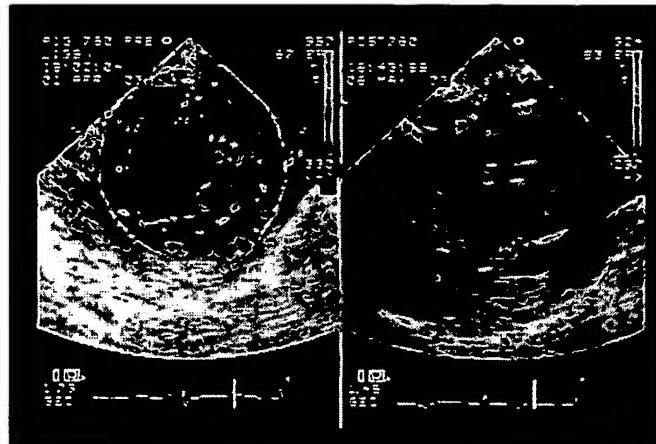


FIG.27A

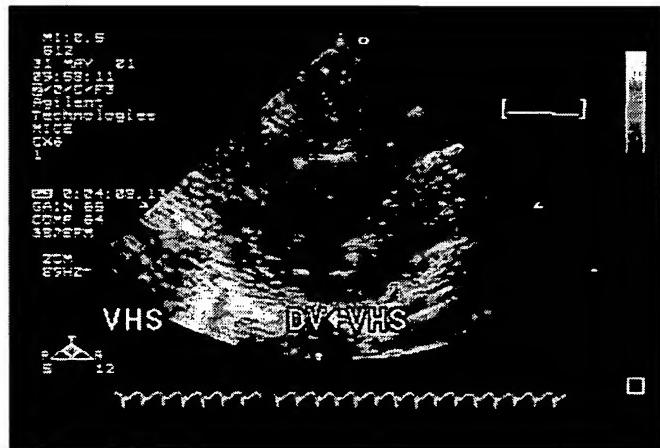


FIG.27B

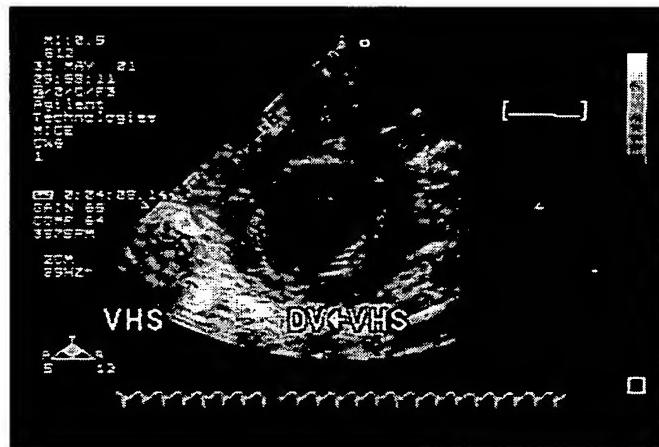


FIG.27C

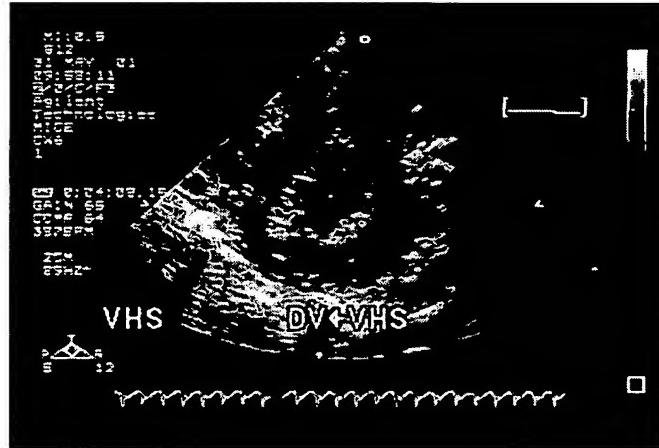


FIG.28

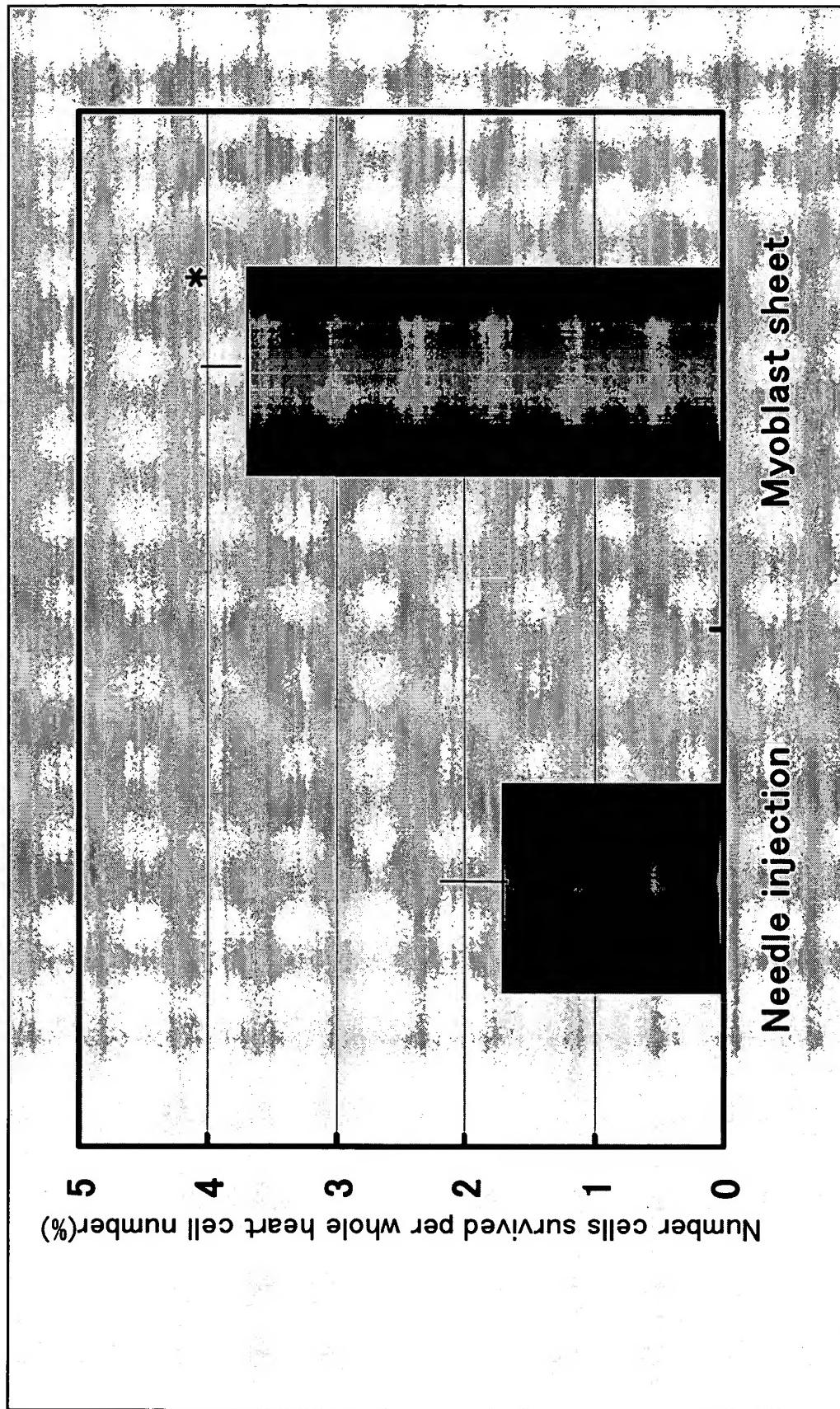
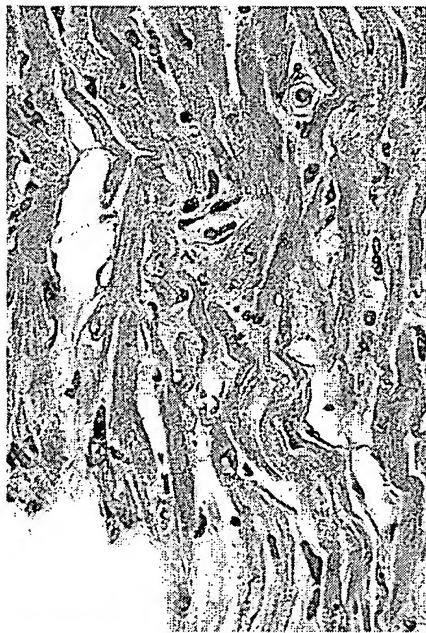


FIG.29

Masson's Trichrome staining x400



HE staining x400



MHC fast x400



MHC slow x400

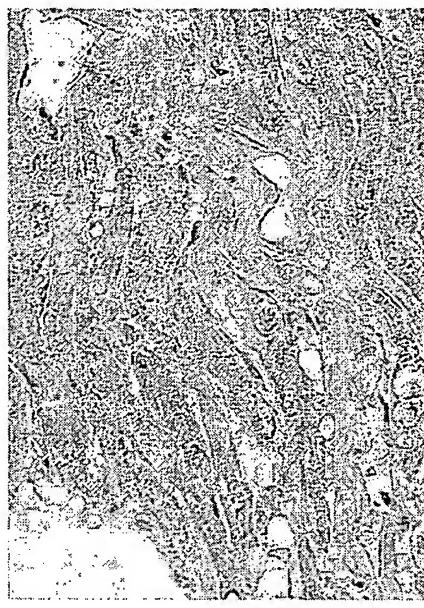


FIG.30A Tissue (Masson's Trichrome staining)

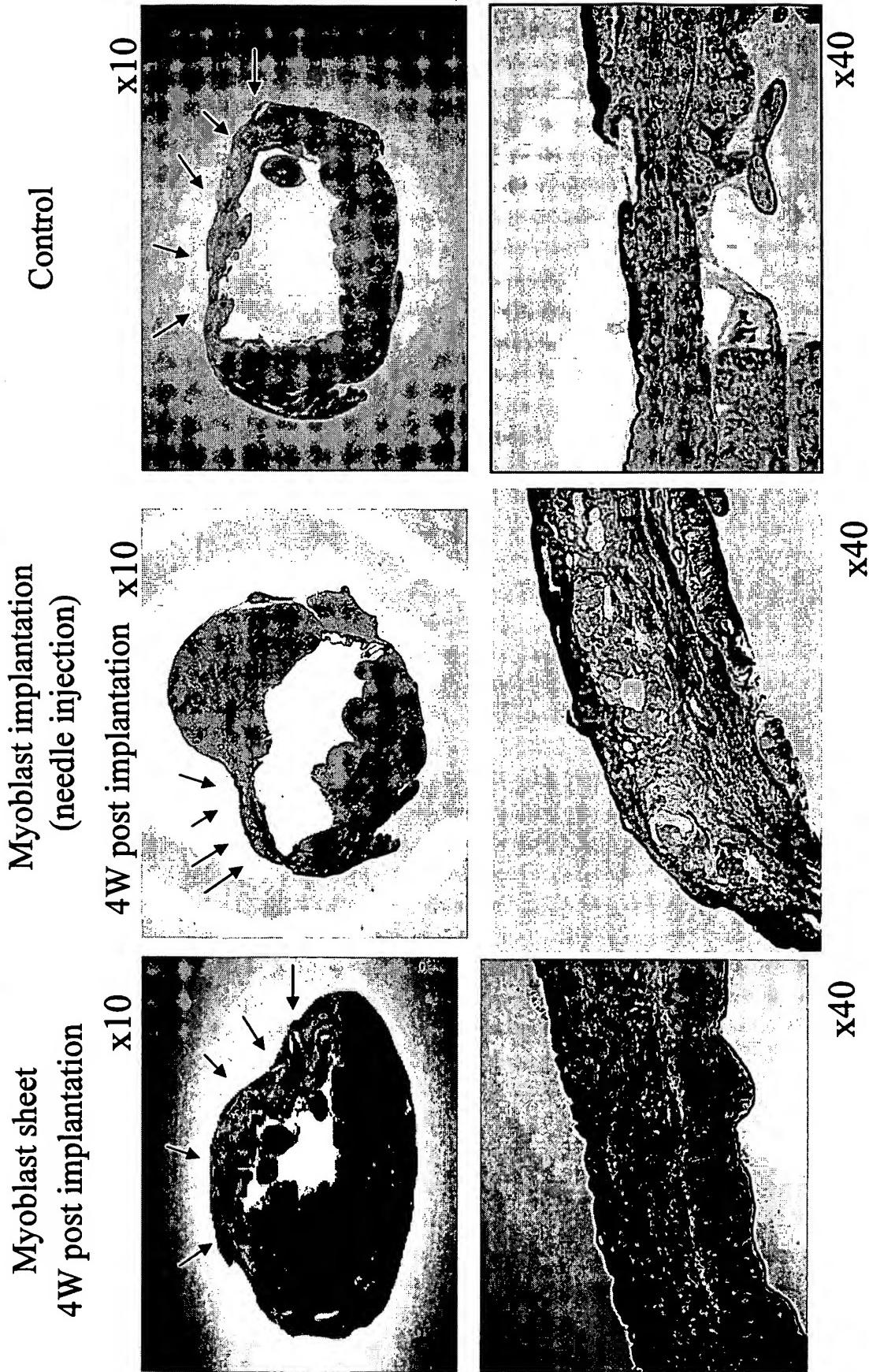
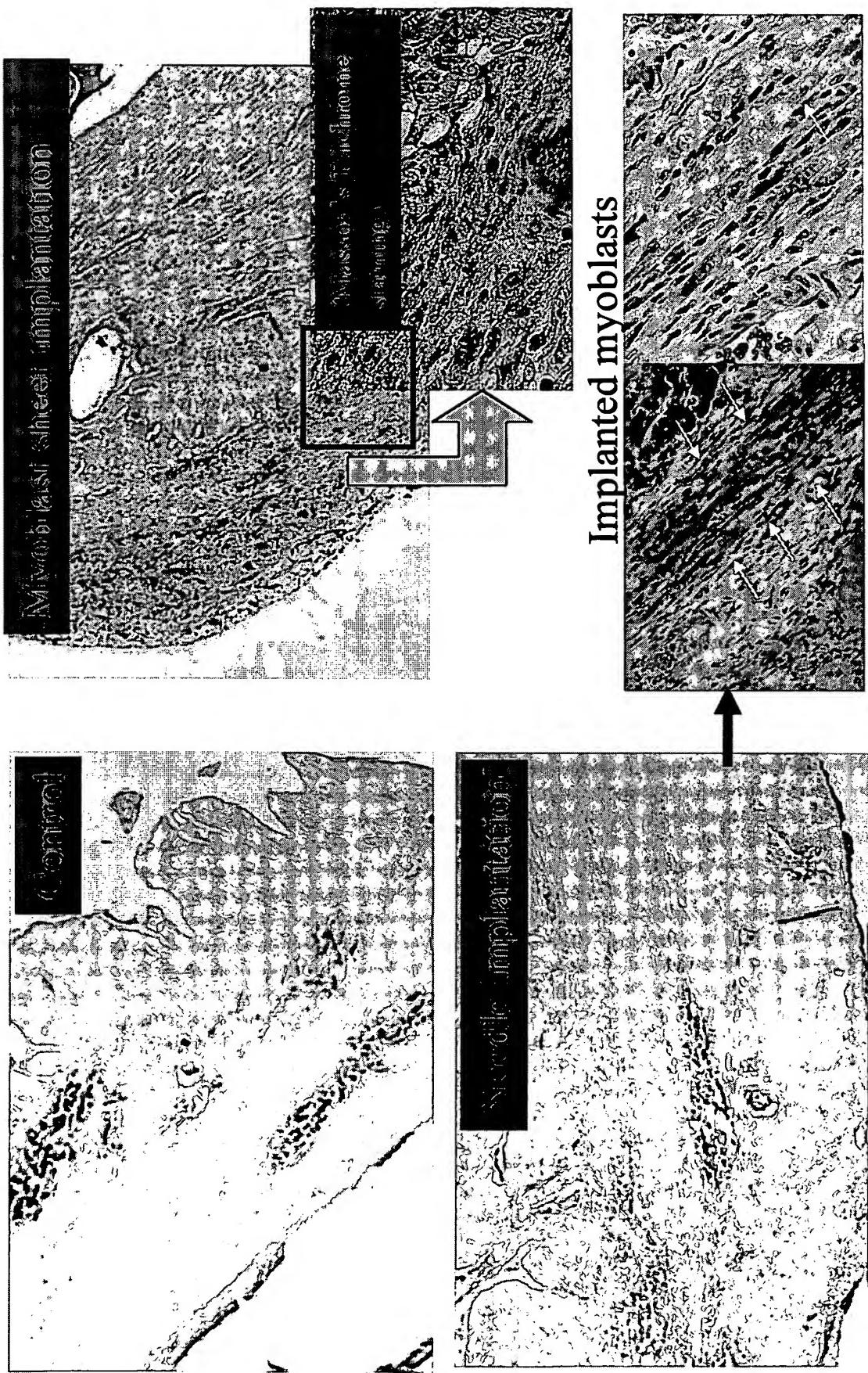


FIG. 30B



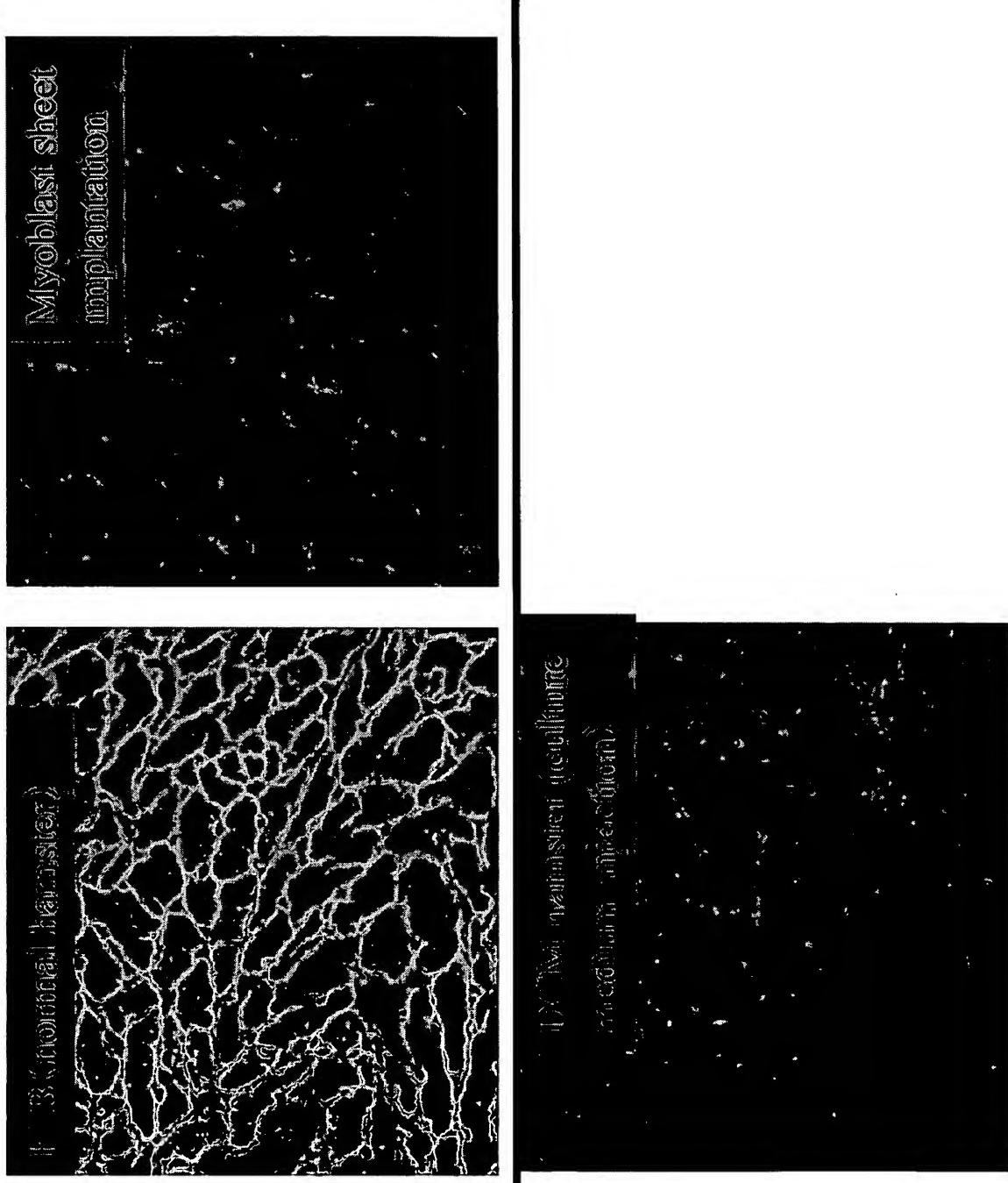


FIG. 30C

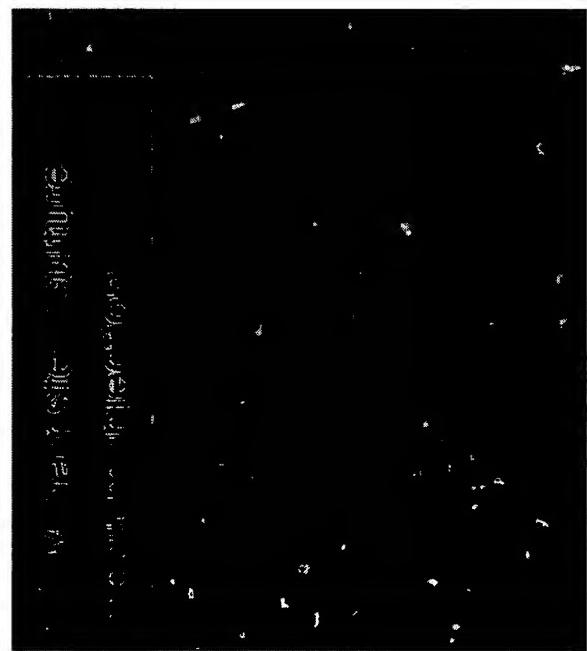
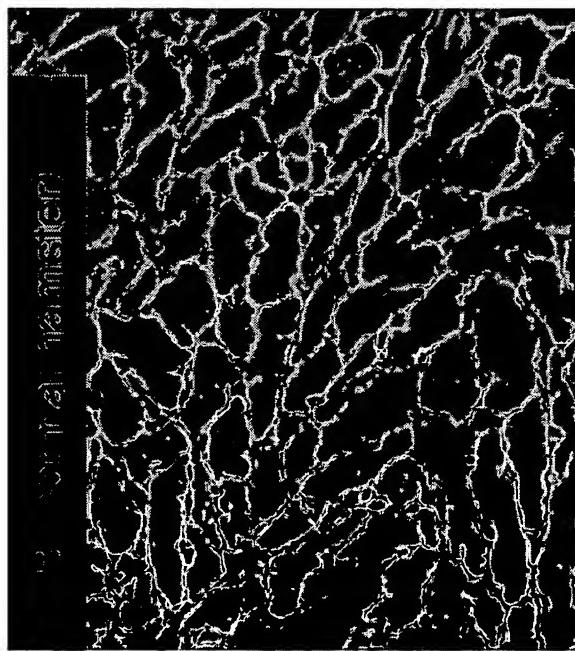


FIG.30D

FIG.31 Survival rate of implanted cell

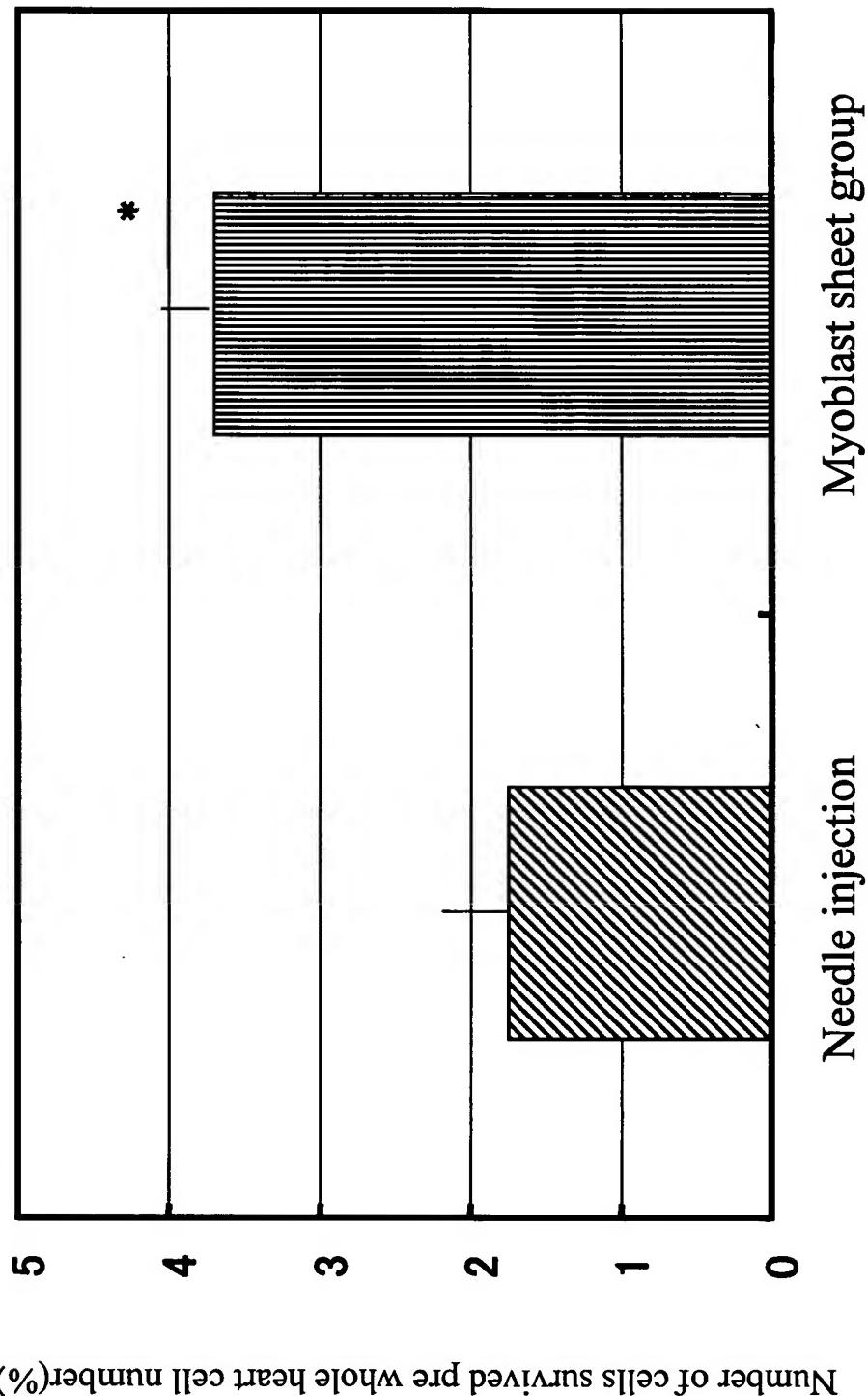


FIG.32 Electronical properties of myoblast sheet

MED system

Cardiomyocyte sheet

Myoblast sheet

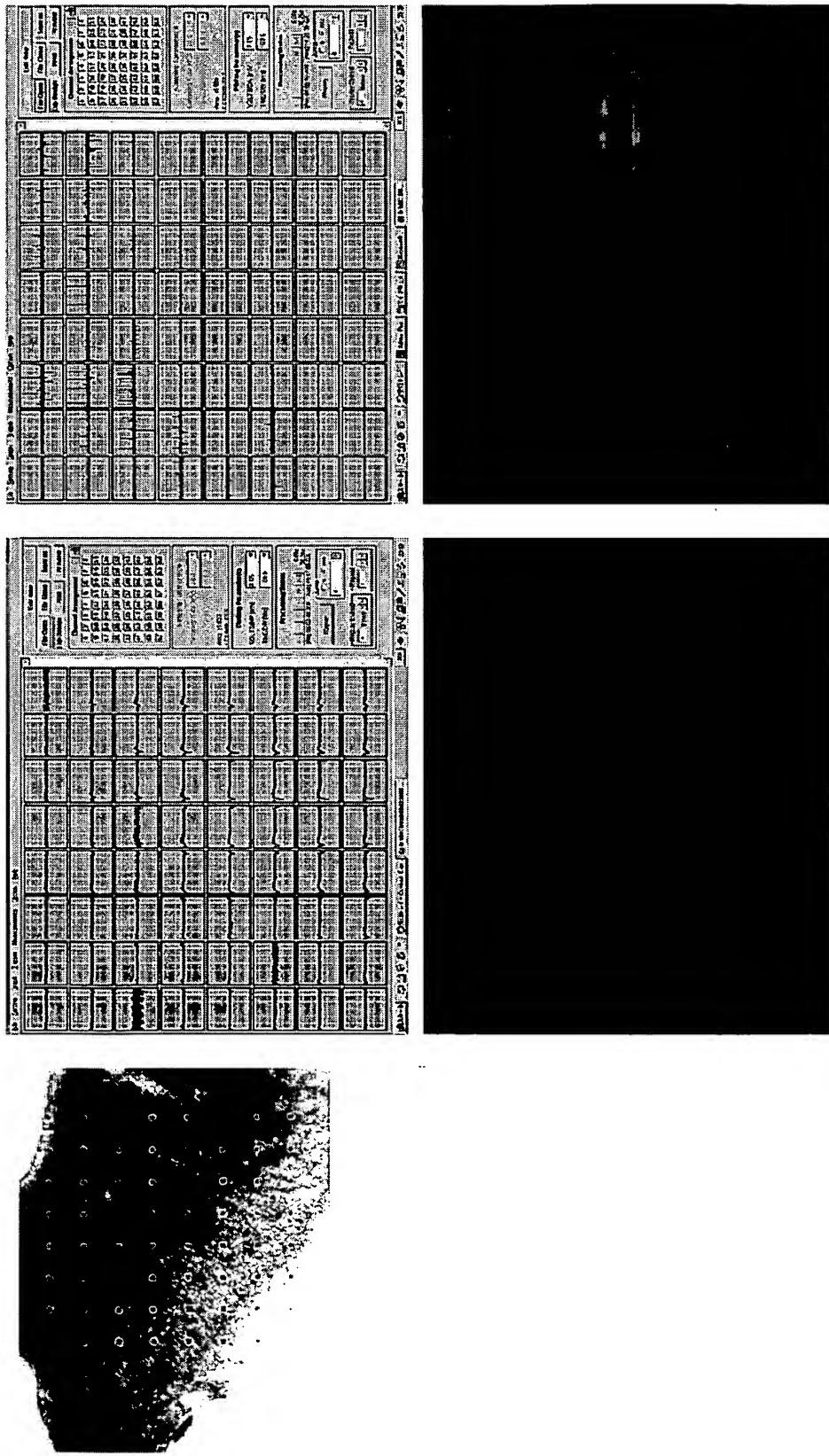
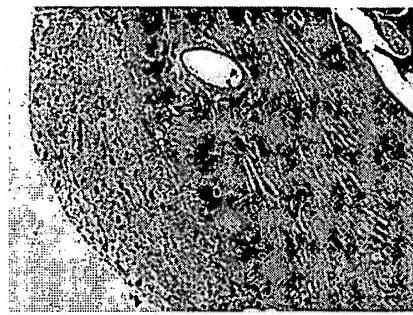


FIG.33A Myoblast sheet implantation to dilated cardiomyopathic hamster

HE staining



Masson's Trichrome staining

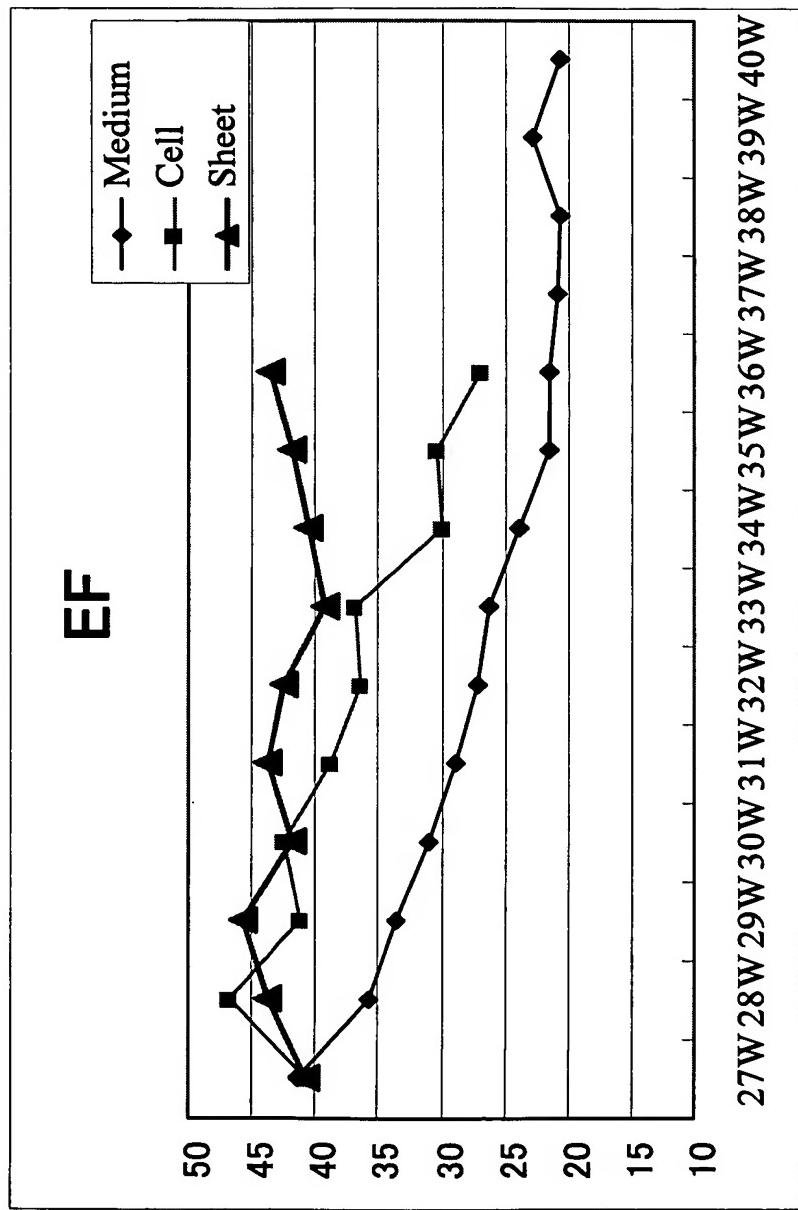
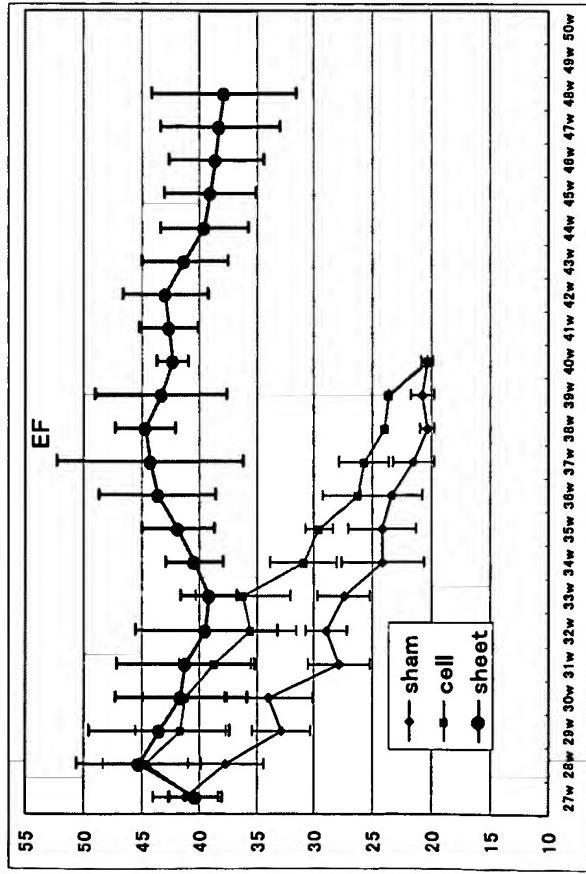


FIG.33B

Left ventricular end-systolic diameter



Left ventricular end-diastolic diameter

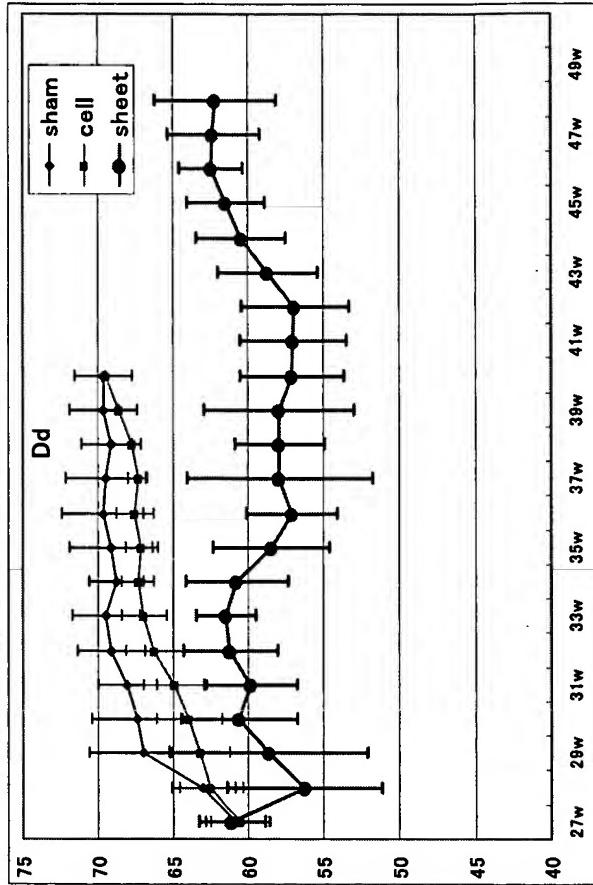
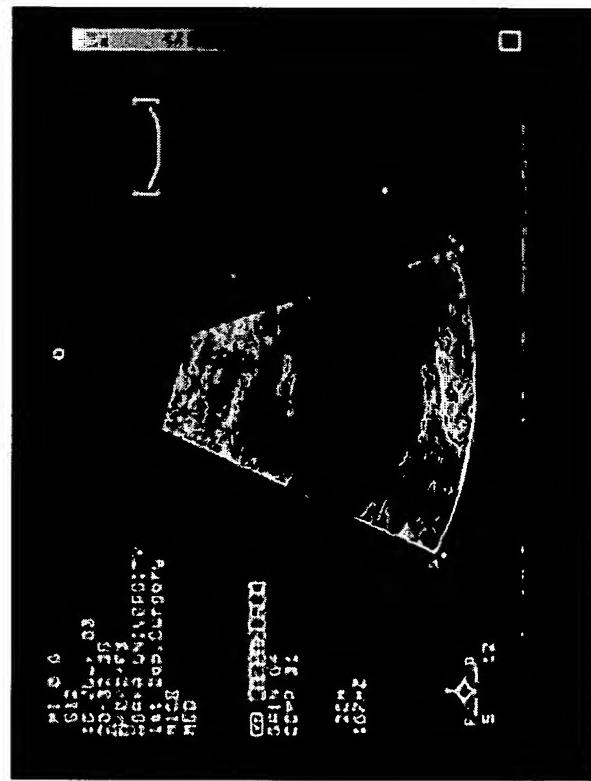


FIG.33C

Control group



Myoblast sheet implantation group

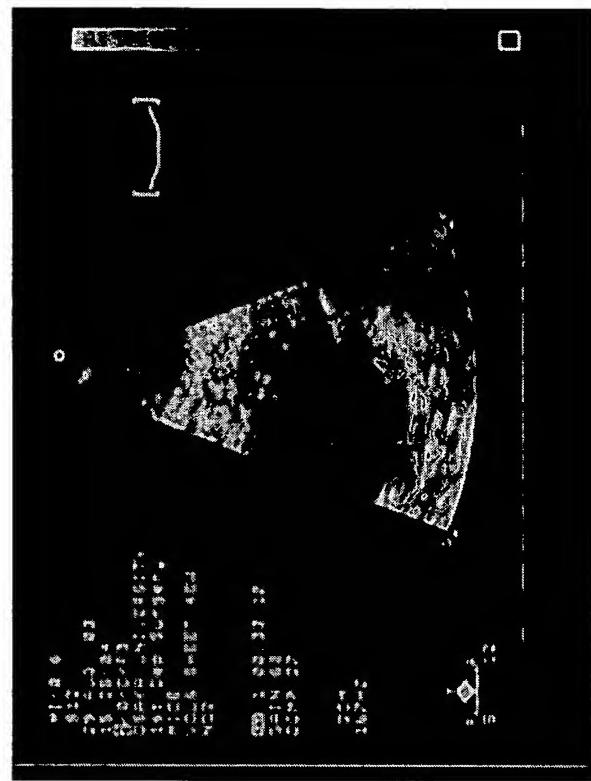
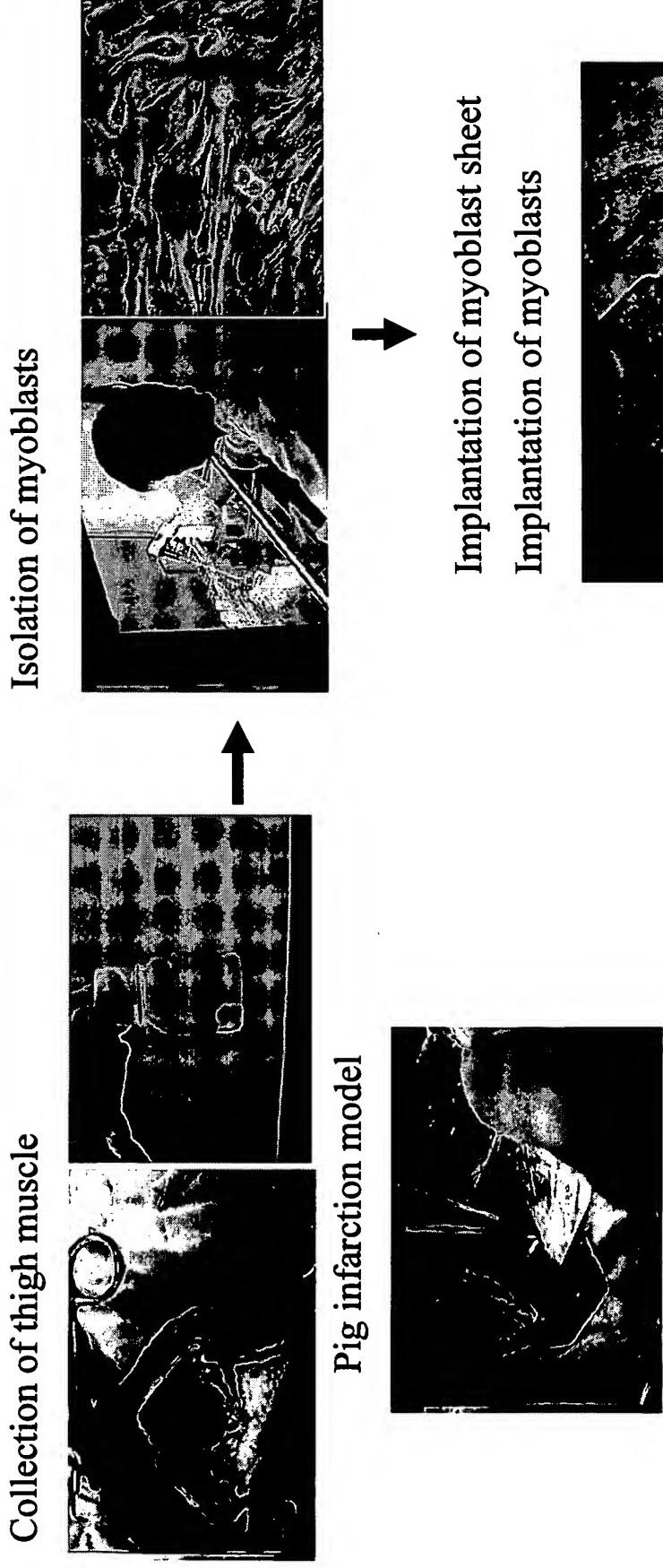


FIG.34 Myoblast sheet implantation into pig infarction model



Evaluation of cardiac function (systolic function) of pig infarction model by CKI method

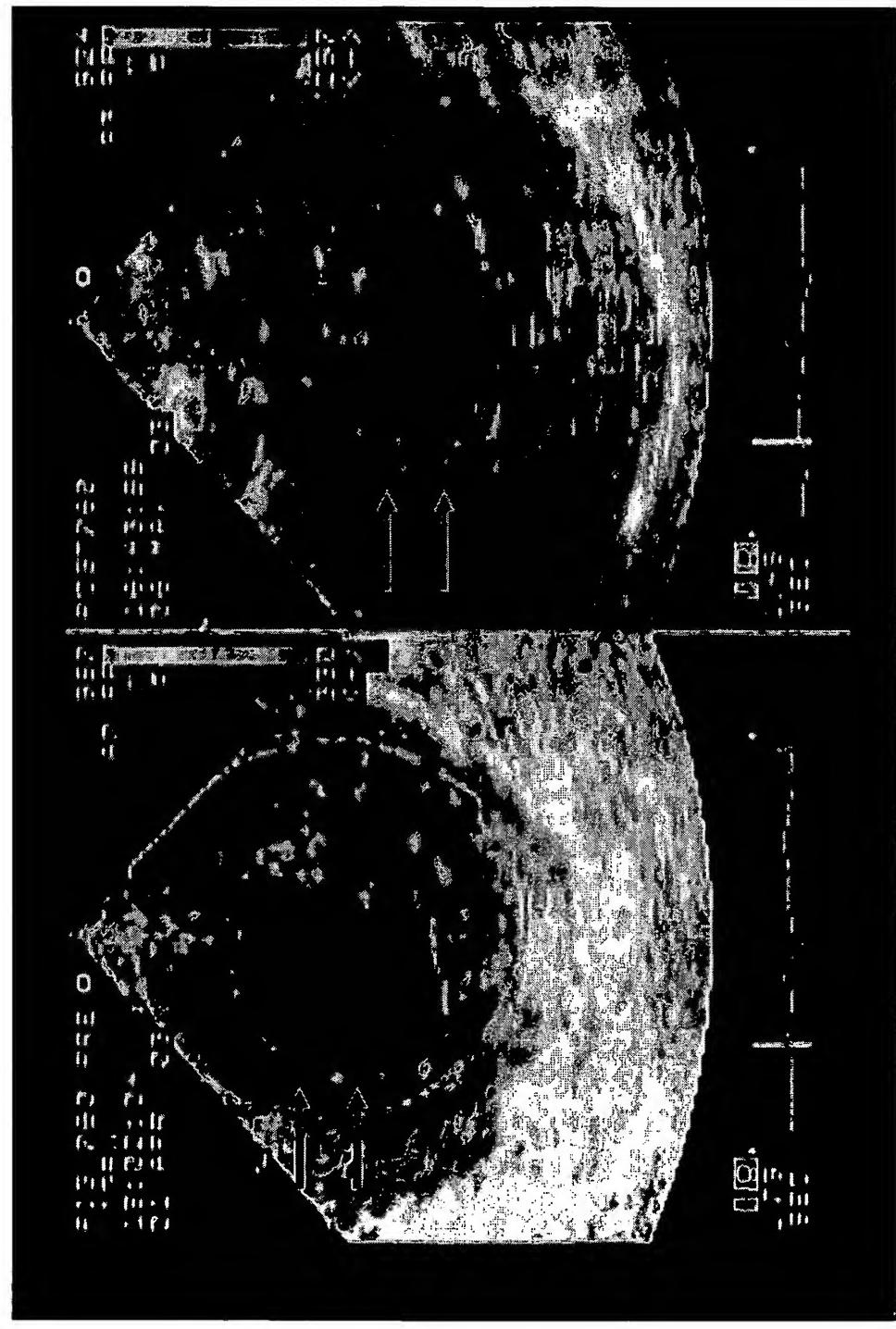
FIG. 35

Before operation After operation



Implantation
site

FIG.36 Evaluation of cardiac function (diastolic function) of pig infarction model by CKI method



→
Implantation
site

FIG.37

Without ascorbic acid



FIG.38

With ascorbic acid

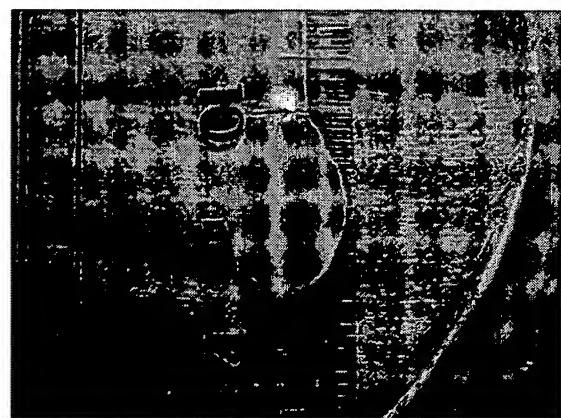
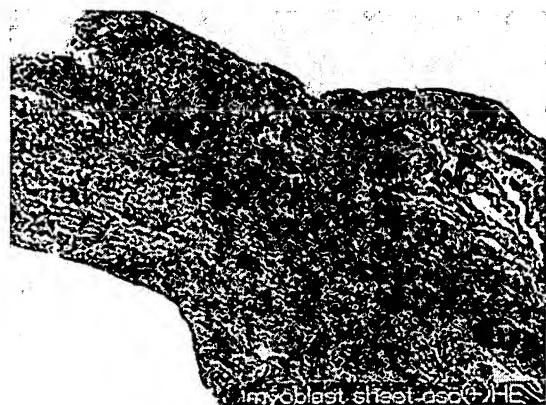


FIG.39



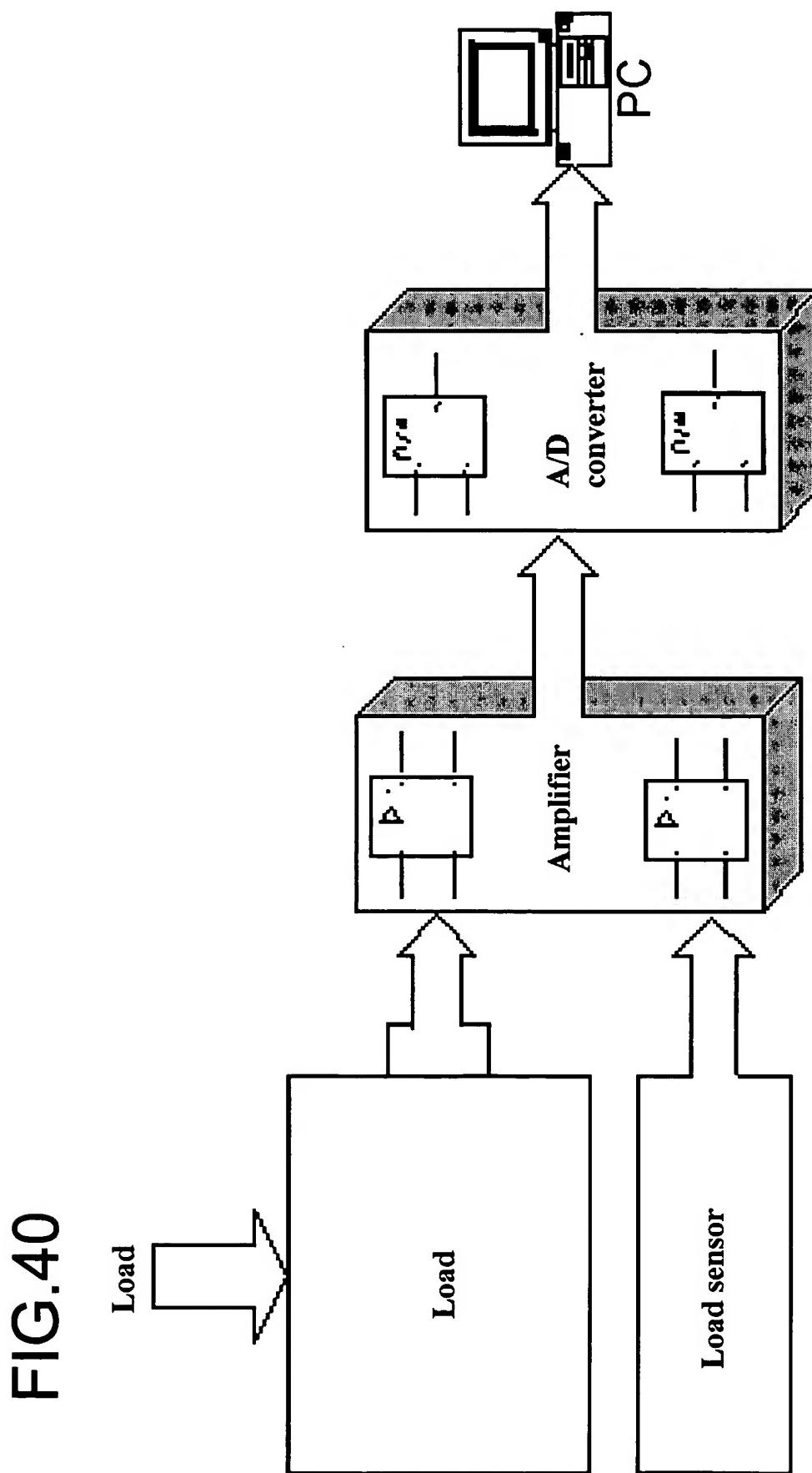
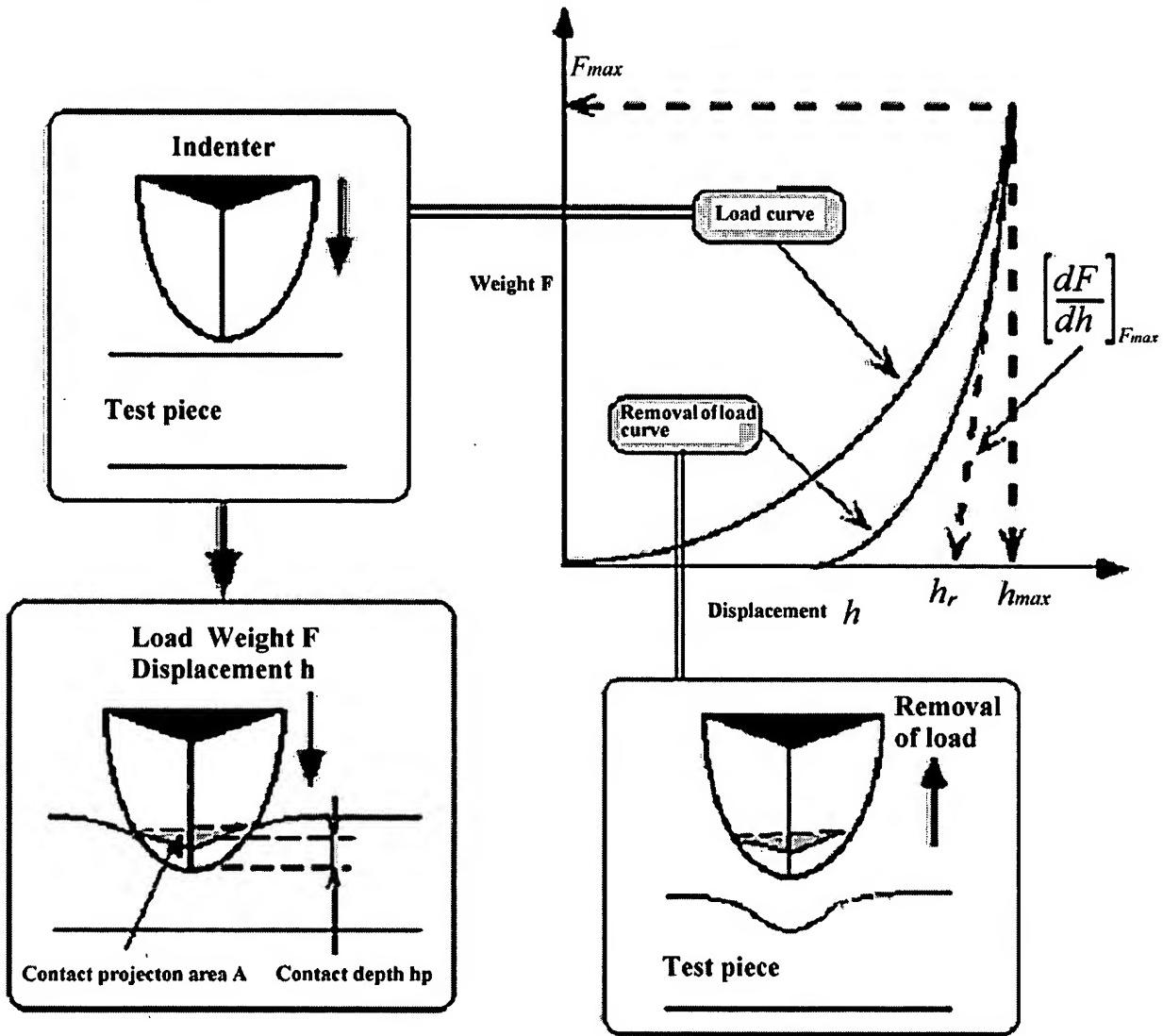


FIG.41



$$\text{Rigidity } H = \frac{F}{A} = \frac{F}{k_1 h_p^2}$$

$$\text{Young's modulus } E = \left[\frac{dF}{dh} \right]_{F_{max}} \cdot \frac{1 - \nu^2}{2 \cdot k_2 \cdot h_{pmax}}$$

$$\text{Contact depth } h_p = h_r + 0.25(h_{max} - h_r)$$

F : Load
A : Contact projection area
h_p : Contact depth area
$k_1 k_2$: Shape coefficient
F_{max} : Maximum load
h_{max} : Max. displacement
h_r : Point at which tangential line cross weight 0
dF/dh : Gradient of tangential line of the removal of load curve
ν : Poisson's ratio

FIG.42



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